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Peace is too politicised for prizes

THE award of the Nobel Peace Prize to Academician Andrei Dmitrievich Sakharov took nearly everyone by surprise. No doubt he could at one time have been considered a candidate for a Physics Prize, but few had thought of him in the context of a Peace Prize. So was the action of the Oslo committee an inspired gesture aimed at expanding the horizons of 'peace' to include human rights as the "only sure foundation for a genuine and long-lasting system of international cooperation"—as the citation read; or was it simply a political act, such as can be carried out from the relative safety of Scandinavia, designed to cause a certain amount of embarrassment to the Soviet Union?

First, let it be said that Sakharov's record, initially as a physicist, then as a proponent of restraint in weaponry and finally as a social democrat fighting for civil rights has been admirable (see page 528). If the year's award is controversial, the controversy is not centred around the personal qualities of Academician Sakharov in any way. Furthermore, it is undeniable that the award of this prize will have done much to encourage Sakharov, if encouragement were needed, to continue his work.

So far so good. But even if the West can, on the whole,

view the award as given for human rights work, the Soviet Union undoubtedly views the award as a piece of political cynicism supportive of troublemakers. If the Nobel Foundation were a political outfit devoted to the supporting of Western ideals that would matter little—but Nobel Prizes would then have the significance only of Stalin Prizes. By aspiring to have a global significance Nobel Prizes must be free of the sort of ambiguity and polarisation that this year's prize generates; and not only this year's—recent awards to Willy Brandt, Le Duc Tho and Henry Kissinger have all in their different ways been political and arguable.

If this means that it is impossible to find globally acceptable recipients of Peace Prizes, then perhaps this is simply a recognition of the fact that the concept of peace is highly politicised and ambiguous, and it is poorly served by the handing out of large sums of money to individuals.

We have noted before that Nobel Prizes often leave a trail of devastation behind them. This is particularly so of the Peace Prize; it is time that the Nobel Foundation seriously looked at other ways of supporting its very worthy ideals.

Better news from the universities

EVEN the most bashful of Britain's universities are now back in business for a new academic year and, according to figures released last week, the population of full-time students (including graduates) is up 10,000 to 261,000. First-year students are up in number by 7%. The reason for the continued growth (albeit at a slower pace than was once predicted) is not entirely that there is a still-growing demand for more higher education among the nation's young. Many of this year's intake will be somewhat reluctant students; the economic situation is persuading many school-leavers that three years at university would be safer than unemployment at 18.

In the past two years universities have been in the forefront of the government's cost-cutting campaign and as a result are now 5% worse off in real terms than they might have expected to be. Sir Arthur Armitage, Chairman of the Committee of Vice-Chancellors and Principals, in announcing these figures, called the meeting of increased demand and the maintenance of university education under these circumstances "no mean contribution to public policy". Few will disagree with him, and

many will hope that the so-called fat-trimming exercise is now at an end.

Behind the overall figures are two statistics; one encouraging, one less so. Even though there are still 10,000 empty science places, there is good evidence that the swing away from the natural sciences has been halted. On the other hand the postgraduate scene is still very static and there might even in the near future be such a thing as a shortage of people with postgraduate degrees. The proportion of overseas students in the postgraduate population remains high.

With 300 university teaching posts now frozen for lack of funds, it is incumbent on universities to enunciate a policy for when (if ever) there is a thaw. There has been a strong element of chance in the freeze—departments with staff at retirement age have been particularly vulnerable. But it is not necessarily logical simply to put the same posts back on the market. A crisis of this dimension offers an excellent opportunity for change in a system normally gripped by tenure. It would be foolish to throw up such an opportunity.



THE 30th Report from the Select Committee of the House of Lords on the European Communities is a slender pamphlet which at first glance seems too trivial to merit a column in *Nature*. It comments on two draft directives from the EEC: one about pollution on bathing beaches (an unseasonable topic anyway, at this time of year), and the other about pollution from wood pulp mills, of which there are only seven in Britain.

In fact the report is anything but trivial. It raises a matter of high principle in Britain's relations with the EEC, affecting our whole policy for the control of pollution. On October 13, the House of Lords "took note" of the report and there was a short debate on it. The debate ought to go on outside Parliament as well.

The matter of principle is this. The Council of the European Communities agreed, in a declaration on November 22, 1973, that "improvement in the quality of life and the protection of the natural environment are among the fundamental tasks of the Community". Admirable. No-one would dissent from this. It follows that there must be a policy among the member states for the abatement of pollution. This policy is now being worked out through numerous draft directives from Brussels prescribing standards for car emissions, lead in petrol and crockery, sulphur in oils and, most recently, the pollution per tonne of product from pulp mills and the quality of water in which bathing is "authorised or tolerated".

The philosophy underlying these directives is to fulfil the 1973 Declaration by imposing identical and uniform standards upon all member states, from Italy jutting into the Mediterranean to Scotland jutting into the Atlantic, irrespective of the diversities in the environments of these states. There is a Gallic elegance and simplicity about this, but it is quite inconsistent with the pragmatic policies which determine the procedures for pollution control in Britain. Do we stick to our present practice and persuade the EEC to accept it? Or do we submit to these inflexible directives from the Community?

Before arguing this point we have to distinguish three kinds of standard, for this controversy with the EEC is concerned with only one of these three kinds. There is a good case for uniformity over product and design standards (for example, the level of pesticide residues in foodstuffs and the emission standards from car exhausts) because food and cars move from one part of the Community to another. There is also a case, provided it is not made too rigid, for long term objectives for the purity of the air and of

the sea. The controversial question is whether there should be Europe-wide identical standards for the quality of emissions into the air from smoke stacks and of effluents into rivers and the sea from outfalls. In other words, must specifications for the discharge of wastes into the environment be identical irrespective of the capacity of the environment to disperse or dilute the wastes?

The practice in Britain is empirical. The consents to discharge effluents, issued by a Regional Water Authority, depend on the capacity of the river or estuary, the uses to which it is being put, the pollution load it is already carrying and so on. The licence to discharge "noxious vapours" into the

A community of interests?



ERIC ASHBY

air, issued by the Alkali Inspectorate, depends on the best practicable means for controlling the vapours at the time the works were erected, the cost of installing new equipment, the economic state of the industry and so on. In brief, the British "philosophy" (if one can call it that) is not to minimise pollution, nor to bring all discharges of any pollutant down to some uniform level (though there are guidelines for quality standards for emissions): it is to optimise pollution levels so that the capacity of the environment to accept (and in many cases to recycle) pollutants is fully used, but not abused, and industry (and thus employment) are not unduly put at risk. (Rabid conservationists need to be reminded, sometimes, that all pollution except that from atomic weapons is a by-product of processes which benefit society.) This "philosophy" can be defended on two counts: it makes the best use of the limited resources available for the control of pollution; and it acts (as the Alkali Inspector put it in a report published in 1888) as "an

elastic band ever tightening as chemical science advanced". Thus in the sulphuric acid industry in Britain there are plants emitting 5%, 4%, 2% and now 0.5% of the sulphur burnt. These different levels are related to the best practicable means for control at the time the plants were built. Had we been obliged to adopt a uniform emission standard it would either have been too lax for new works or it would have added unreasonably to the costs of running older works.

Consider now the EEC's draft directive for effluents from wood pulp mills. It lays down that within 10 years pollution shall be reduced to levels set out in the directive. These prescribe the biological oxygen demand (BOD) and suspended solids (SS) per tonne of product, above which discharges from mills will not be permitted. There is an escape clause which allows derogations from these standards if the discharge is into tidal waters, provided the discharges "do not cause an appreciable deterioration in the quality of the receiving water" ("appreciable" is not defined!). But the derogations expire after a maximum of 10 years. So this is what the directive means: that ultimately the permitted effluent standard from a wood pulp mill shall be identical, whether the mill discharges into the Thames or the Rhine or into the Atlantic Ocean off the Scottish or the French coast.

It requires no subtlety to see that this sort of rigidity would be a wasteful way to deploy the limited resources we have for the abatement of pollution. But the EEC has another reason for pressing uniformity on member states. It asserts that if the Scottish mill discharging into the Atlantic can get away with less stringent effluent standards, this will constitute unfair competition against the mill on the Rhine, which has to comply with very stringent standards. This is a phoney argument, for you might as well say that the wage rates and the transport costs for the Scottish mill will constitute unfair competition unless they, too, are identical with the rates and costs for the mill on the Rhine. "Harmonisation" of practices (as the EEC calls it) among member states is obviously a sensible objective, but it becomes irrational if it disregards the great diversity of environments in the Community.

The draft directive for the quality of bathing water is unacceptable for a different reason. It is not that we are content to bathe in dirtier water than our common market cousins bathe in; it is that a code of control which might be appropriate on a Mediterranean beach at a temperature of 27°C would be inappropriate on an Aberdeenshire beach at a temperature

(under favourable conditions) of 10 °C. The EEC directive goes into great detail about the monitoring of beaches where bathing is to be authorised or tolerated. (How does one deal with a bather who persists in bathing in a place which is not tolerated? The directive is silent on this point.) Monitoring must be carried out for each beach once a week, or once every two weeks, or once a month, depending on "the mean density of bathers per kilometre of beach" and bathing is defined as "prolonged immersion of the whole body". Bathing water is "deemed to conform to the mandatory values of the relevant parameters if 95% of the samples . . . comply with the limits specified in column I of annexes 1 and 2". Now turn to the list of parameters in the annex. They include: total coliform, faecal coliform, faecal streptococci, *Salmonella*, viruses, pH, mineral oils, surface active substances reacting with methylene blue, phenol indices, pesticides, and the following: As, Cd, Cr, Pb, Hg, CN, NO₃, phosphates and dissolved oxygen. Consider the task of carrying out these acts of monitoring, even for the major watering places round the coasts of Britain. The Department of the Environment hazarded a guess that the cost of complying with the directive might be of the order of £100 million a year. If there were a demonstrable health hazard this enterprise might be justified, but such data as we have (from a Medical Research Council report published in 1959) indicate that although many beaches in Britain are offensive to the eye and the nose, they are not a hazard to health; and the WHO still takes the view that "there are no internationally accepted criteria for the quality of coastal water for bathing with respect either to microbial contamination or chemical pollution".

There are no grounds for complacency about the state of the environment in Britain. We are certainly not in disagreement with the EEC's Declaration to protect the environment and we have, in the Control of Pollution Act, the best legislative arrangements in Europe for doing the job. The matter of principle, on which I hope Britain will stand firmly, is that each member state should be free to fulfil the objectives of the Community's environment policy in the light (as the Declaration states) "of health and ecological requirements"—that is to say, taking into account the uses to which the environment is being put, and its capacity to dilute or disperse wastes. This is the basis of our pragmatic style of pollution control. This is the style we shall have to abandon if we cannot persuade the EEC to make its directives more flexible. □

Candle power at Browns Ferry

In his third article on the state of the US nuclear industry, Colin Norman recalls the bizarre and dangerous accident at a nuclear power station earlier this year.

A FIRE, started by an electrician with a candle, disabled the Browns Ferry nuclear power station in Alabama on March 22, 1975, and sent shock waves through the nuclear industry. Officially described as the most serious accident to occur so far at an operating nuclear power plant in the United States, the fire knocked out several key safety systems, caused problems in shutting down one of the plant's two reactors, and rekindled the long-smouldering debate about nuclear safety.

A bizarre and dangerous accident which destroyed some 2,000 electric cables and caused millions of dollars worth of damage, the fire has put the Browns Ferry power station out of action for an indefinite period. It has also raised a number of serious questions about the vulnerability of nuclear power plants to such unforeseen incidents, and it may result in some costly modifications to other nuclear power stations in the United States.

The nuclear industry has, however, managed to salvage some good news from the incident. Although several key safety systems were disabled by the fire, enough reserve systems were left intact to shut the plant down safely and to avoid a major accident. That, industry spokesmen suggest, supports their contention that there are so many safety features built into a nuclear power station that if one device fails, others will be left to take over if necessary.

The Nuclear Regulatory Commission (NRC), the agency responsible for regulating the nuclear industry and ensuring that nuclear power plants are safe, shares that interpretation. In testimony before the Joint Committee on Atomic Energy last month, for example, the Chairman of the NRC, William A. Anders, said "we believe that this unfortunate and serious occurrence has shown that the reliance on the defence-in-depth concept is sound for the protection of public health and safety".

The NRC is now in the midst of analysing the Browns Ferry fire and its implications. It has already published a factual report on the accident, and in the next few weeks, the NRC will

recommend changes in design and operating procedures at other nuclear power stations to prevent similar incidents from occurring elsewhere. Its report on the fire itself is a disquieting document which reveals an astonishing lack of fire precautions, considerable confusion during the fire fighting operations, and some quick thinking in the control room to avert a major nuclear disaster.

The fire began at about 1215 on March 22, when two reactors at Browns Ferry were each generating about 1,100 MW of electricity. A third reactor, of similar size, was under construction. The fire was touched off by two electricians who were testing for air leaks around cables passing through the wall of a room enclosing the Unit 1 reactor.

The air in the reactor room is maintained at a lower pressure than the air outside, so that if radioactivity were released from the reactor, it would not immediately be dispersed into the atmosphere—fresh air would tend to leak into the reactor room rather than contaminated air out of it. The electricians were checking for leaks in a part of the wall separating the reactor room from a cable spreading room, immediately beneath the reactor's control panels. They were using the highly unofficial but time-honoured method of holding a lighted candle to the outside of the wall at the point where cables pass through it—if the candle flame is sucked horizontally, air is leaking from the cable spreading room into the reactor room.

An electrician's mate, who was doing the candle work, told NRC investigators that a fire started when the candle flame was sucked into a hole in the wall and ignited polyurethane foam surrounding some cables. He tried to beat out the smouldering foam with a flashlight, but succeeded only in burning the flashlight lens. He then tried to smother the fire with rags, but that didn't work. Somebody brought him a carbon dioxide fire extinguisher, but the CO₂ blew straight through the hole without extinguishing the fire. Fanned by the air rushing through the hole, the fire began to get further back into the wall. He tried firing off two dry chemical fire extinguishers, but without success, and about 15 minutes after the fire started, the evacuation alarm sounded in the cable spreading room where he was working.

The cable spreading room was evacuated in preparation for triggering the permanent carbon dioxide fire extinguishing system. In the meantime, another electrician raised the alarm in a roundabout way (the correct fire alarm telephone number was not listed in some plant emergency manuals, so there was some confusion)

and the reactor operators in the control room were notified of the fire. By that time, the burning had travelled through the hole in the wall into the reactor room, where several cable coatings had caught fire. Firefighting efforts there were hampered by the fact that the burning cables were about 40 feet above floor level. The local fire department was notified at 1309 and fire trucks arrived at 1330 from Athens, about 10 miles from the plant.

Among the alarming features of the firefighting effort noted by NRC investigators were the following:

- The carbon dioxide system initially failed to work because the power had been shut off and metal plates had been placed over the manual cranks to guard against accidents during construction work.

- Smoke in the reactor building was so dense that the room was evacuated at about 1300, and "there appears to have been no central organised direction of the fire fighting efforts in this area until approximately 4.30 p.m."

- Soon after he arrived at the plant, the Athens fire chief recommended spraying the fire with water, but the plant supervisor refused to allow use of water because of the danger of electrical shorts and injury to the fire fighters. He eventually sanctioned the use of water at about 1900 and the fire was extinguished within 15 minutes. It was formally declared to be out by 1945. By that time, the fire had damaged cables about 40 feet into the reactor room and a few feet inside the cable spreading room.

In contrast to the firefighting efforts, attempts to shut down the reactors were more successful, but no less dramatic. Because some key equipment had been disabled by the fire, the reactor operators were forced to use what the NRC coyly describes as "unconventional" methods to shut one of the reactors down and to avoid a potentially serious situation.

The reactors are controlled in a common control room immediately above the cable spreading room. The operator in charge of the Unit 1 reactor started noticing strange behaviour of various indicators on his control panel about five minutes after he had been notified of the fire. He was getting erroneous indications that pumps were running, some pumps cut in automatically, power output from the reactor began to drop, and several lights on the control board began glowing abnormally bright and then getting dim or going out. At 1251, the operator decided to shut the reactor down by inserting the control rods into the core, thereby cutting off the chain reaction (in operator's parlance, he manually 'scrammed' the reactor).

That successfully halted the nuclear

reaction, but the problems then began. Because radioactivity decay of fission products in reactor fuel continues to heat the core long after the chain reaction is shut off, cooling water must be circulated through the core for many hours afterwards. Unless adequate cooling is provided, the reactor core could melt, burn its way through the concrete and steel floor of the pressure vessel and release large quantities of radioactivity into the environment. Because means of supplying cooling water to the reactor were knocked out by fire, the operator in charge of Unit 1 had to improvise.

Among the most important systems which were disabled was the emergency core cooling system (ECCS), a device which is supposed to flood the core with water if a pipe ruptures and the main cooling water is lost. The ECCS is used routinely during reactor shutdown operations.

According to the NRC report, and a description of the event provided to the Joint Committee on Atomic Energy last month by Donald F. Knuth, Director of the NRC's Office of Inspection and enforcement, the following sequence of events took place after the reactor was manually scrambled.

The water level surrounding the core dropped as a result of reduced boiling when the chain reaction was shut off. Consequently, pumps designed to inject water at high pressure into the reactor vessel increased their flow rate to keep the core covered with water. Soon after those pumps began running, however, a steam valve closed and disabled them. At that stage, the NRC report states, there was the following dangerous situation:

"The reactor coolant system had one remaining source of high pressure water, the control rod drive (CRD) system. Operator M (who was in control of Unit 1 operations) increased the CRD pump output to its maximum. Although the control room instrument has a maximum scale reading of 100 gallons per minute, Operator M stated that he knew that the CRD pump was pumping greater than 100 g.p.m. but he does not know how much was being pumped. Operator M advised the investigators that he did not think that starting the spare CRD pump would have resulted in significantly greater injection flow, and he recalls that the spare CRD pump was not always operable during the shutdown".

In other words, there was only one reliable means of injecting high pressure water to the reactor, and that was being strained to its limit to maintain the coolant level around the core.

It soon became apparent that the SRD pump system couldn't maintain

water level. It was therefore decided to open relief valves to reduce pressure in the reactor so that low pressure pumps could be used to supply cooling water. That tricky operation was carried out at about 1330, and the low pressure pumps provided sufficient water until about 1830, when control over the relief valves was lost and they slammed shut. Pressure in the reactor began to build up, and the low pressure pumps were rendered inoperable.

The operator was then forced to switch back to the CRD system to supply high pressure water. That arrangement provided sufficient coolant until about 2150, when control over the pressure relief valves was restored and the reactor was again depressurised. About 13 hours after the fire began, temporary repairs were completed to allow the reactor to remain at low pressure, and at about 0430, normal means of cooling the core were restored. By a series of extraordinary procedures, which have won praise from the NRC investigators, the reactor operators were therefore able to shut the plant down safely. But the incident nevertheless raises some very tricky questions.

Aside from the astonishing comedy of errors which led to the fire and caused it to burn for nearly 7 hours when it could have been extinguished with water in a few minutes, inadequacy in the design of the Browns Ferry plant was highlighted by the accident: a single fire, in a relatively small area of the plant disabled several key safety systems. Could a similar fire at another plant also have such potentially disastrous consequences?

Investigators from the NRC are now looking into the implications of the fire for other plants. According to Dr S. H. Hanauer, who is directing the investigation, important cables in plants built since Browns Ferry are more widely separated so that fewer key systems would be disabled by one fire. But "further improvements may be prudent in the light of the Browns Ferry lessons", Hanauer suggested, and he added that "some rerouting of cables may be necessary in some existing plants". Nevertheless, Hanauer stated that "based on our evaluation of the incident, we believe that even if a fire such as the one at Browns Ferry occurred in another existing plant, the most probable outcome would still be with no adverse effects on the public health and safety". The investigation, Hanauer said, "has not shown that present power plants are unsafe".

But the Browns Ferry fire had the makings of a very serious nuclear accident, and it has already provided plenty of ammunition for nuclear critics to shoot at the nuclear industry's otherwise good safety record. □

THE deficit in this year's grain harvest can hardly have come at a more embarrassing time for the Soviet planners. Just when internal propaganda is aimed at high output and increased productivity, at greeting the forthcoming Twenty-fifth Party Congress in a spirit of "socialist emulation" and celebrating the fortieth anniversary of "Stakhanovite" work, the Soviet government has had to admit, at least at the international level, that in spite of an ever-expanding programme of the mechanisation and electrification of agriculture, the harvest has once again failed to meet the basic requirement of feeding the Soviet population.

Scanning the Soviet press, it is difficult at first glance to find evidence of the deficit. On October 3, when the Soviet government was already seeking abroad the grain which it had failed to produce at home, *Radyans'ka Ukrayina* reprinted a speech of V. V. Shcherbyts'kyy, First Secretary of the Communist Party of Ukraine, which claimed: "Twenty regions have already fulfilled the agricultural plan [for grain delivery] ... and nine regions have overfulfilled the firm plan." Similar news has peppered the Soviet press throughout August and September. Collective farms, state farms, districts and regions have been reported, one after another, as fulfilling the plan.

Yet when one reads more carefully, a somewhat different picture emerges. Immediately before announcing his apparently promising picture of overfulfilled plans, Shcherbyts'kyy had admitted that hopes for a good harvest had fallen victim to "extremely unfavourable weather conditions", but that nevertheless, the total yield would be "considerably higher than in 1972" (cold comfort, since 1972 was the year when crop failures resulted in considerable food shortages). Later in the same speech, he mentioned the importance of guaranteeing adequate supplies of animal feed—implying that this year's reserves were dangerously low, if not actually insufficient. It may well prove necessary to slaughter off a considerable number of cattle on the collective cattle-rearing farms.

It should also be remembered that even if grain production quotas have been fulfilled, in the sense that the requisite amount has been delivered to the official collecting points, a bad harvest may mean that a collective farm is left with no surplus to be shared out for the support of its members and their domestic livestock for the coming year, causing the farm members to become, temporarily, consumers rather than producers, and necessitating a further depletion of

the country's livestock. Shcherbyts'kyy's speech refers, of course, only to Ukraine, but reports from the other grain areas of the Soviet Union, notably Kazakhstan and western Siberia, paint a similar picture.

The emphasis on delivery to the state of the prescribed quotas is fundamental to Soviet agricultural policy. In the early days of collectivisation, this led to considerable wastages. Forbidden by Draconian legislation to reap any grain for their personal use before the plans were fulfilled, peasants had to let their horses and oxen starve for lack of feed-

Alien corn?

from Vera Rich

stuffs, and so had no beasts left to draw the reapers when harvest could officially commence. Although such a situation would hardly be likely today, the precarious balance of Soviet agriculture, officially based on collective or state farms, but in reality deriving a considerable proportion of its vegetables and other small scale products from the personal allotments which the farmers cultivate in their free time is still liable to break down under any unusual conditions. Press announcements that peasants have been stealing "collective" grain to feed to their "private" cow or chickens can hardly account for deficits of millions of tonnes, but do, in some way, symbolise the whole problem of Soviet agriculture. The collective/state farm system simply cannot meet the demands made on it. At the popular level, the solution is that proposed by the Twenty-Fourth Party Congress: more mechanisation, more extensive use of fertilisers. The theoretical journals, however, show a deeper concern.

Last year, writing in *Planovoe khozyaistvo* (No. 3, 1974) the Chairman of the State Planning Commission noted a recent falling off of theoretical and methodological work on balancing the economy. Several follow-up articles in the same journal show considerable concern with the balance of the agricultural and industrial sectors. Similarly, writing in *Voprosy ekonomiki* (No. 8, 1975), M. Bronshtein, Corresponding Member of the Academy of Sciences of the Estonian SSR made an extensive analysis of the "Economic and Social Problems of the Industrialisation of Agriculture". The word "industrialisation" is significant here—Soviet planners see agriculture as being essentially one more branch of industrial production, amenable to the same system of fulfilment and overfulfilment of plans. The present trend towards

specialisation and concentration of different branches of the "industry" in different areas often, however, takes too little account of the fact that agriculture is what Bronshtein calls a "biomechanical system". His article is tentative and theoretical, but it does reflect a certain awareness that agriculture has its special problems which will not necessarily respond to an industrial-type treatment.

Although such discussions do take place at the theoretical level, however, Party policy demands the acceptance of the fundamental superiority of the Soviet agricultural system. On October 7, the Deputy Minister of Agriculture, A. A. Gol'tsov made a statement in passionate defence of the system. Although the final figures are not yet in, he said, an "average" harvest is expected. The Soviet Union is one of the world's leading grain producers. Grain yields are well up to international standards. Export and import of grain is "normal practice" in international trade. Grain purchases from the USA are intended for the Soviet Far East, to obviate the long haul across the Trans-Siberian railway. The Soviet Union not only imports grain but also supplies it to other countries. Protested far too much, he then drew attention to the "steep growth" in agricultural production which has taken place since the 2.3 million tractors and 600,000 combines of today replaced the 17 million wooden harrows of Tsarist times. He stressed the great social benefits that collective farm life has brought to the peasants. Unfortunately, his argument breaks down on one vital fact—Tsarist Russia was a major grain exporter, the "breadbasket of Europe."

Four days later, addressing a rally of collective farmers, the Minister of Agriculture, Dmitrii Polyanski, spoke far more realistically, admitting that "unusual weather conditions" would inevitably be reflected in the general level of agriculture as a whole. It is one thing, however, to admit a sudden emergency, but another to suggest that the system itself might be at fault. For this reason, although in the present atmosphere of detente the Soviet government is entering into all manner of trade agreements with Western and Third World countries, even in such sensitive areas as oil production or nuclear fuels, there has so far been a considerable reluctance to make some firm agreement on grain purchases. Present negotiations, although described as being in a "very delicate stage", seem, however, to be moving towards the concept of a regular-sales agreement. The stabilisation of the world grain market thus produced would be of long term benefit to both parties.

international news

Sakharov and the cause of peace

from Vera Rich, London

THE announcement last week of the award of the 1975 Nobel Peace Prize to Academician Andrei Sakharov coincided neatly with the 250th anniversary celebrations of the Soviet Academy of Sciences. This event, however, was not claimed by the academy as a culminating point of the celebrations, a tribute, as it were, to the efforts of Soviet science in the cause of peace. For the very activities which have won Sakharov the Peace Prize have brought him into disfavour with the Soviet establishment, and although he still retains his status as an academician, he does so on sufferance.

Sakharov's involvement in problems of peace and human rights has arisen as a direct corollary of his scientific career. After defending his PhD thesis (on cosmic-ray theory) in 1947, and after publishing only three scientific papers he disappeared from the world scientific scene into a top-secret establishment working on the development of the H bomb. At this stage in his career, Sakharov was convinced that a balance of power was the only sure guarantee of world peace and, "carried away by the immensity of the task", he contributed several key ideas to the research, although he has always eschewed the title of "father of the Russian H bomb" conferred on him by Western journalists, stating that the project was essentially one of "collective invention".

At the same time, together with Igor Tamm, he began working upon non-military aspects of thermonuclear reactions and plasma physics. It was for his work on the bomb, however, that he received (secretly) a Stalin Prize, three Orders of Socialist Labour, and a salary of 2,000 roubles a month, with numerous fringe benefits, such as access to restricted consumer goods, a chauffeur-driven car and a round-the-clock bodyguard. His election, in 1953, as a full member of the Academy of Sciences, without passing through the intermediate stage of corresponding member, was likewise a result of the military aspect of his work.

During the 1950s, however, Sakha-



Sakharov and his wife, Yelena Bonner: academician on sufferance.

rov's attitude to the nuclear arsenal began to change. He became increasingly concerned with the genetic and pollution effects of nuclear fallout, and also with the tremendous consumption of resources and manpower involved in making bombs. From 1958 onwards, he campaigned consistently against nuclear tests—earning from Khrushchev the comment that Sakharov was a good scientist, but should leave politics to the politicians. This, however, Sakharov was not prepared to do, and the campaigns continued. Finally he was able to urge the acceptance of a test-ban treaty covering the atmosphere, space and the ocean.

In 1964, Sakharov became involved in the great debate of the Academy of Sciences and the Academy of Agricultural Sciences which, after a quarter of a century of Lysenkoism, led to the reinstatement of genetics as a respectable branch of Soviet science. This event, apparently only an internal matter of academic policy, was to have great psychological significance for Sakharov. By now he was experiencing a complete revulsion from the whole concept of nuclear weaponry. He was increasingly disturbed by the ethical problems engendered by the bomb and by the often cynical attitude of politicians towards such problems. At this point, as a result of his participation in the Lysenko debate, he became acquainted with the biologist Zhores Medvedev, and through him with his

twin brother, the historian Roy. The Medvedev brothers introduced Sakharov to *samizdat*, the growing body of clandestine, self-published typewritten literature, which circulated among the growing circle of those who would later be called dissidents. In 1968, Sakharov made his own contribution to *samizdat* literature with a monograph entitled *Progress, Coexistence and Intellectual Freedom*.

This manuscript circulated widely in *samizdat* and inevitably reached the West, where it received considerable publicity. The Soviet government accordingly revoked Sakharov's security clearance, and it was almost a year before he could find another post—as a "senior researcher" at the Lebedev Physics Institute, the lowest grade of post in which an academician can legally be employed. Two years later, Sakharov published a second "manifesto", which was also signed by Roy Medvedev and a physicist, Valentin Turchin. This took the form of an open letter to the Soviet leaders, claiming that the major internal problems of the Soviet Union, the slowing down of the economy and a failure to meet the challenge of the "second industrial revolution" of the computer age could only be met by increased democratisation.

From now on, Sakharov was to be increasingly concerned with the problems of the individual. In November 1970, he formed, with Andrei Tver-

dokhlebov and Valerii Chalidze, a Human Rights Committee, maintaining that in doing so he was acting within the guarantees of the 1936 Soviet Constitution. Since then he has campaigned unceasingly for the release of dissidents illegally confined in mental institutions, for the rights of Jews to emigrate to Israel, for the abolition of the death penalty, for the rights of minority communities within the Soviet Union, for the amnesty of political detainees. In doing so, he has brought down on himself considerable pressure from the authorities and open attacks in the Soviet press which, in 1973, evoked fears that a show trial might be imminent.

His large personal fortune, amassed during his work on the H bomb, he donated, some years ago, to the Soviet government's Cancer Research fund, and although retaining his status as an academician, the material benefits normally accruing to such a position have steadily vanished. Furthermore, his scientific career has been interrupted by the "inner unrest" and by the increasing demands of all those who have come to regard him as a kind of ombudsman for the dissidents. He admits that he has "not been productive" of recent years, and on occasion has suggested that his most fertile years are behind him. Those who have had occasion to speak with him on scientific topics (mostly young refusniks and others who find it difficult to continue their scientific career under the prevailing conditions) maintain, however, that his insight and scientific intuition is as active as ever and that a conversation with Sakharov can throw a whole new light on their work.

The award of the Nobel Peace Prize to Sakharov may be seen by many to contain a certain paradox. His early involvement in the H-bomb programme and his growing involvement with internal abuses in the Soviet Union rather than with the broad issues of peace between nations—these may seem strange qualifications for a Peace Prize winner. But it was in his involvement with thermonuclear weapons that Sakharov first came face to face with the ethical problems involved in their use; seeing peace and detente as the only alternative to a nuclear holocaust, and believing that the desire for peace is shared by the majority of mankind, he believes that the only sure guarantee of peace is that of a democratically based society, in which governments will permit a free discussion of all issues which concern the community and which, in the words of the Nobel citation "the inviolable rights of man . . . serve as the only sure foundation for a genuine and long-lasting system of international cooperation."

THE business of exporting nuclear technology to countries which are not signatories to the Nuclear Non-proliferation Treaty (NPT) has come in for serious criticism after the 'leaking' of secret documents implicating West Germany in the development of a South African programme capable of supplying nuclear weaponry. In Bonn, members of Chancellor Schmidt's government have spent the past few days issuing denials of involvement, denials of denials and even (from State Secretary Klaus Boelling) a 'correction' of his denial to make it an affirmation that Kraftwerksunion (KWU) had indeed asked for an official guarantee to export nuclear reactors to South Africa.

The row has already led to the resignation of Lieutenant General Gunther Rall, the West German representative on NATO's military committee, who was said to have visited South Africa secretly and under a false name, as a guest and adviser of the South African Defence Department. It has also led to a general reassessment of a policy under which Bonn has agreed, in spite of US opposition, to supply to Brazil a package deal involving a complete nuclear fuel cycle which would allow, if required, the manufacture of weapons-grade material.

The debate has been escalated by the news that Egypt has asked Bonn to cooperate on its nuclear programme, and that South Africa is reported to be planning the sale of 14,000 tons of uranium oxide to Iran in a deal which would give Iran a part share in a large uranium enrichment plant, capable of producing fissionable material and based in South Africa.

One of the most awkward problems in controlling the spread of nuclear weapons is the establishment of a mechanism to ensure that equipment and material supplied for civilian use is not actually converted to a military end. The West German-Brazilian deal brought the problem sharply to the attention of the countries capable of managing such sales (the USA, the USSR, France, West Germany, Japan and Britain) and they are reported to be having confidential talks to draw up a set of rules to govern nuclear exports. Whatever the outcome of the talks (and it is said that a set of safeguards is likely to be agreed by the end of the year) it looks as though the German-South African deal will go ahead, in spite of a rather vague undertaking from Bonn that the political implications as well as the economic will be considered before it agrees to issue a licence to KWU to build reactors for South Africa.

But even if the sale does go ahead, its accomplishment will be attended by a good deal of embarrassment for both the West German and South African governments. And the laying bare of the coy, undercover exchanges by which the two countries corresponded has been a thoroughly successful public relations exercise on the part of the African National Congress (ANC), which was responsible for the exposé.

Documentation for the deal, including the secret telegram which led to the downfall of Lieutenant General Rall, was among material believed to have been stolen by a spy from the South African Embassy in Cologne. It was published by the ANC in an account entitled "The nuclear conspiracy: FRG [Federal Republic of Germany] collaborates to strengthen apartheid".

According to the ANC, a uranium enrichment plant to be built in South Africa next year has been developed with the assistance of the state-owned Gesellschaft für Kernforschung (GFK) and the state-controlled company STEAG, with the agreement and active participation of the Bonn government. The prototype plant, it is estimated, will cost \$1,400 million to install, and an ancillary power station costing a further \$800 million will have to be constructed in order to provide energy for the process.

After making comparisons with the costs of conventional fuel sources, the ANC argues that a uranium enrichment plant in South Africa cannot be justified economically on energy grounds, and can only be explained in political and military terms. As Pretoria has refused to sign the NPT, the ANC claims, it will be able to use the enrichment plant "to produce uncontrolled material for nuclear weapons. The threat of proliferation could be used to deter embargoes and sanctions, while the threat to use nuclear weapons could be used to extend apartheid's hegemony on the continent of Africa. The use of nuclear weapons cannot be precluded as a desperate measure to preserve the apartheid state."

The suggestion is not simply paranoia on the part of a struggling black nationalist group. The South African state-owned broadcasting corporation reported only last week that "more and more countries are acquiring nuclear power stations, and with them the automatic capability of making nuclear weapons", and many of the country's leaders are committed to the development of a nuclear arms capacity as an integral part of a civil nuclear programme. □

THE European Science Foundation (ESF) last week marked its first year of existence with the publication of its first annual report and with a simultaneous venture into the field of public relations. Although it has clearly decided that it is now ready to present itself to the scientific community, the foundation did not have much in the way of positive achievement to report, other than its readiness to tackle the tasks it has set itself. Sir Brian Flowers, the foundation's first president, was, however, able, in part at least, to fend off a good deal of scepticism about the organisation's future effectiveness.

Sir Brian made clear last week that the sole aim of the foundation is to further international cooperation in new scientific schemes. He admitted, however, that little had been done during the past year apart from "organising the organisation", though one or two definite moves have already been made towards coordinating research projects. These include the incorporation into the foundation of the pre-existing European Medical Research Council (EMRC) and Western European Science Research Council (ESRC) and the setting up of an *ad hoc* Committee for Space Science and a Standing Committee on Astronomy. More specifically, the foundation has managed to implement international research projects in fields as widely scattered as genetic manipulation, particle physics, and the preservation of mediaeval stained glass.

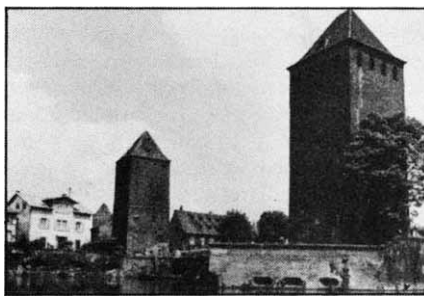
But Sir Brian denied that the foundation will ever be called upon to fulfil any role other than overseeing cooperation in pure research and preventing unnecessary duplication, adding that he saw no need for the ESF to become involved with the "interface between politics and science". From that relatively shaky standpoint it was easy for him to defend the ESF against sceptics expecting an easy time at the expense of an organisation operating on a budget of £200,000 with a full time staff of six based in Strasbourg. Nonetheless, the foundation still has to weather its first storm, and though its board may insist that the ESF can be effective in ensuring that there is no repetition of the situation whereby Britain, France and Germany all expend individual effort on the construction of broadly similar particle accelerators, it remains to be seen what weight the foundation carries when it comes to national chauvinism. At the moment there is optimism that the foundation will be able to influence participant governments through its member organisations—Britain's include the five research councils, the British Academy and the Royal Society—though what would happen, in the absence of finan-

cial control by the foundation, should these organisations themselves revert to nationalistic stances has not apparently been considered by the board.

All the same, Sir Brian's resolution and view of himself as first and foremost a European must count for something, and he instils an undeniable air of confidence throughout the foundation. He insists that the ESF should be run with a human touch, perhaps acceding to the point often made that it was established to pre-empt the bureaucracy of the Brussels administra-

Strasbourg diary

by Allan Piper



tion. Though not in as many words, what he apparently advocates is a return to grass roots policy-making, but within a pan-European framework. In a rare display of emotion last week, he expressed horror at the notion that the foundation should become nothing more than a central computerised clearing house of research data, preferring instead to envisage the setting up of "a little piece of Europe" for the scientific community.

Whether or not that small international community can expect to grow much larger in the near future is on the surface as indeterminate as many other aspects of the foundation. Though the admission to the foundation last Wednesday of the Greek National Hellenic Research Foundation brought the number of member organisations to 43, representing 16 countries, Sir Brian did not evince much enthusiasm for the suggestion that countries from the eastern bloc be admitted. Yugoslavia has, in fact, been a member since the founding of the ESF but Sir Brian feels that, though approaches from other communist countries would not be discouraged, the foundation for its part would be better off spending the next five years or so consolidating its position and will not make any overtures in that direction.

For the more immediate future, the Executive Council of the ESF has just passed fresh proposals recommending research projects involving, among other things, the general place of biology in space research, patterns of

human migration within Europe, the difference between existing European legal systems, and methods of teaching advanced mathematics. That selection identifies the eclectic scope covered by foundation, and illustrates the point made by everybody connected with its running that the word "science" should be interpreted in a broad European sense of knowledge or learning. Whether or not such an essentially academic approach will be able to survive in the latter half of the twentieth century only time will tell; in the interim, it is as well to share the optimism of the ESF representative who feels that when heads need knocking together, as they surely will, Sir Brian Flowers is the right person to do it.

● Out on the eastern edge of the city, in a solid concrete building unflatteringly reflective of a more substantial international role, the Parliamentary Assembly of the Council of Europe (CE) was last week convened for a nine-day plenary session. Ample evidence there of the political-scientific interface mentioned by Sir Brian Flowers. Essentially a political organisation the council has set up a European Joint Committee of Scientific Cooperation (EJCSC) comprising a mixture of scientists and parliamentarians engaged in the running of research groups and working parties. One obvious advantage which the council has over the ESF lies in the political muscle it has developed over 26 years of existence; thus, any findings coming out of the EJCSC may eventually come up for consideration by the council's Committee of Ministers, so that pressing issues of practical concern stand some chance of being satisfactorily resolved at an international level. During last week's session, for instance, the issue of pollution of the Rhineland water table came up for discussion. The problem is that the water table, lying below the surface, provides 80% of the water reserves for an area that covers parts of France, Switzerland and Germany, so that both the consumption and the pollution of the water transcend national boundaries. In the past there has been little attempt at international control but by bringing the issue to the attention of the CE a coordinated approach should be ensured over the coming months. It may seem invidious to compare the workings of a small, newly formed organisation expressly unconcerned with the problems of technology, with a well established political body working on a multimillion pound budget, but the comparison does illustrate that, whatever else the ESF may aver, politics and science are becoming ever more inseparable.

● There was an unmistakable air of satisfaction during the session over the

THE Natural Resources Defense Council (NRDC), which has long been a prominent thorn in the flesh of the nuclear industry, has now launched a drive for stricter radiation standards in the United States. In petitions filed during the past two weeks with the Nuclear Regulatory Commission (NRC) and the Environmental Protection Agency, NRDC staff scientists Arthur Tamplin and Thomas Cochran argue that workers exposed to radiation at the present maximum permissible levels run an unacceptable risk of dying from cancer and of having children with serious genetic defects. Consequently, they argue that the permitted exposure levels, at least for younger workers, should be reduced by about a factor of 10.

On the basis of several reports and studies published in the past few years (particularly a 1969 report by the International Commission on Radiological Protection and a report on the biological effects of ionising radiation (BEIR) published in 1972 by the National Academy of Sciences), Tamplin and Cochran have calculated that between 2 and 6% of all radiation workers exposed to whole-body radiation at maximum permissible levels for their working lives could die from radiation-induced cancer.

Though they point out that few radiation workers are in fact exposed to the maximum permissible levels of radiation, Cochran and Tamplin suggest that such a risk is "excessive" and that the standard is an "inappropriate guideline" for the nuclear industry.

Similarly, the petition contains calculations suggesting that workers in the nuclear industry who are exposed to maximum permitted levels of whole-body radiation would stand between a 1 in 10 and 1 in 300 chance of having children with serious radiation-induced genetic defects.

The present maximum allowable whole-body dose of radiation is 5 rem a year, and no more than 3 rem in one calendar quarter. Cochran and Tamplin argue that the standard should be replaced by a requirement that workers under the age of 45 should receive no more than 0.5 rem a year to the whole body, and not more than 0.3 a quarter.

Older workers would be allowed higher radiation doses—up to a maximum of 3 rem a quarter, however.

A two-tier exposure level, Cochran and Tamplin argue, would offer the greatest protection to those most susceptible to radiation injury, such as pregnant or fertile women, while not at the same time applying the strictest standards to other parts of the workforce. "Hence, the proposed changes

Washington seen

by Colin Norman

should not place a large burden on the industry", they argue.

If the NRDC's proposals were adopted, the greatest impact would be felt in fuel-reprocessing and plutonium-fabricating plants, but no such facilities are yet in commercial operation. The immediate effect of the petition, however, is likely to be to reopen the long standing debate about the consequences of long term exposure to low levels of ionising radiation.

● Following recent revelations that the Central Intelligence Agency (CIA) and the Department of Defense have been carrying out ethically bankrupt research on people—such as feeding psychotropic drugs to soldiers without their knowledge or consent—Senator Edward Kennedy has proposed a bill to establish a Presidential commission to oversee all federally supported clinical studies.

The bill would essentially transform a panel which was established by Congress last year to draft regulations governing human experimentation supported by the Department of Health, Education and Welfare, into a permanent body with broad responsibilities. Called the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, the panel has already issued recommendations for research on fetuses, and it is now trying to draft guidelines for research on prisoners, children and the mentally ill. It is expected to complete its work early next year.

Kennedy said last week that the abuses perpetrated by the CIA and the Department of Defense "underscore

the need to expand the national commission's jurisdiction, and to expand it now". His bill would add the Secretary of Defense, the Secretary of Health, Education and Welfare, the Director of the CIA, four Senators and four Representatives to the panel's 11 existing members. It would also establish the commission as a permanent advisory body with responsibility to make public recommendations on clinical studies sponsored by all government departments.

Since Kennedy is the chairman of the Senate Health subcommittee, to which the bill was referred, and since it is also backed by two senior republicans on the subcommittee—Jacob Javits and Richard Schweiker—it stands a very good chance of being passed by the Senate.

● The latest in a long line of statements by prominent scientists warning about the dangers of nuclear power was made public with considerable fanfare last week. It is particularly noteworthy for two reasons. First, it resulted from a study sponsored by the National Council of Churches—a body with considerable influence. And, second, it was supported by a galaxy of scientific stars, including 14 Nobel Prizewinners.

Called "The Plutonium Economy: A Statement of Concern", it warned about the dangers of allowing plutonium to be extracted from spent reactor fuel rods and recycled as a nuclear fuel, and it spoke out against the development of the fast breeder reactor. "We believe that the proposed 'plutonium economy' is morally indefensible and technically objectionable", the statement said.

Drafted by a committee chaired by Rene Dubos and Margaret Mead, and supported by a lengthy background report on the dangers of plutonium, the statement was requested by the National Council of Churches last year. After remarking on the dangers of using plutonium, the report concludes that "all who believe that technology should serve human values should join in opposing the plutonium economy and in seeking to divert into more constructive channels the vast resources being devoted to nuclear power."

council's role in the setting up of the recently formed European Space Agency (ESA). As long ago as 1960 the council was calling for one European agency to handle the building of both launchers and satellites, and the amalgamation of ELDO and ESRO last May was regarded by most delegates as the results of persistent lobbying by the council. The feeling of achievement was celebrated last week by the assembly when it unanimously

agreed a draft recommendation that member states of ESA meet at least once a year at ministerial level. The feeling is that ministerial meetings will provide the agency with an impetus which might otherwise be lacking.

American confidence in the agency has been demonstrated by a request for the ESA to cooperate in NASA's post-Apollo programme: a space laboratory is to be built by European industry under the guidance of the agency.

Apart from scientific and technological benefits, a fully independent ESA could also provide industry and employment—there is even a whisper that the agency ought to become outwardly commercial and seek to establish new markets within the Third World. One delegate, perhaps mindful of the approach of 1984, even expressed relief that with a fully independent space industry Europe will be in the running for worldwide communications control.

correspondence

First-year ecology

SIR,—Although I sympathise with many of the sentiments expressed by Dowdeswell and Potter (October 2) it seems to be that some of their difficulties could be overcome by giving up the search for "natural" habitats (which hardly exist in Britain) and instead concentrating on the immediate surroundings. Take, for example, the ordinary suburban garden. Its high diversity of plants supports an immense variety of animals (most of them insects) and an array of ecological situations reminiscent of tropical rain forest. Virtually all the principles necessary for a first-year ecology course can be demonstrated and investigated in a garden. There is no shortage of material and an imaginative ecologist is soon able to find examples of what he wants.

There are populations of aphids, ladybirds, earthworms, and woodlice for practical work in population ecology. There are intricate food webs centred around every plant and tree. The onion bulb fly, the two-spot ladybird, the peppered moth, and a host of others, can be used as examples of the gene as an element of continuity in populations. I think that the trouble is that rather little is known and published about the ecology of gardens. Research money, as we all know, has gone to the study of "natural" areas and farmland and gardens have been dismissed by some ecologists as biological deserts, which is a pity, for here is a vast expanding resource invaluable to teacher and conservationist alike. Indeed the fact that so little is known should be exploited in the development of a spirit of inquiry among first-year students. Finally, although I would not dispute the value of simulation experiments and visual aids I remain sufficiently old-fashioned to believe that direct contact with nature is better.

D. F. OWEN

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Deterioration

SIR,—Whereas the quality of the scientific contributions to *Nature* remains uniformly of the highest calibre, I have noticed over a period of several years a steady deterioration in the quality of editorial contributions.

A recent example (August 21) is the

astonishing revelation that the old Chinese had discovered relativity, galaxies, perhaps pulsars and X-ray stars. Surely the author should re-read his classics to find out that, far from being 1,300 years behind, western thought was in fact several hundred years ahead of these Chinese speculations.

In Lucretius's *De Natura Rerum* (about 55 BC), for example, we find also ideas about empty space ("All nature as it is in itself consists of two things—bodies and the vacant space in which they are situated", Book I, 418) and about time and space ("It must not be claimed that anyone can sense time by itself apart from the movement of things", Book I, 460). All this was preached by Democritus in the fifth century BC, but many other examples of anticipations could be found in the writings of Greek philosophers.

I fear that the review article in question is indicative of a spirit of masochistic depreciation of science in general and Western culture in particular, which is regrettable in the offices of so prestigious a scientific journal. Many readers will also remember that ample space was given to an Indian journalist for a vicious attack against the USA and the WHO, and that only several months later did the editor feel obliged to shed some crocodile tears in an editorial more designed to whitewash himself than to put things right.

Yours faithfully,

Dr S. V. VAECK

Hofstade, Belgium

Collectors' Code

SIR,—In recent years there has been a good deal of comment on the remarkable zeal shown by some American bird and egg collectors, which led to much discussion at the XVI International Ornithological Congress in Canberra last year (*Nature*, 248, 543; 249, 793; *New Scientist*, 64, 734; 1974). The leading American learned society, the American Ornithologists' Union, set up an *ad hoc* committee under the eminent conservationist John Aldrich to consider the whole matter, and they have now produced a lengthy report.

After emphasising the number of birds killed by hunters and pest-control agencies and the need for scientists to have adequate freedom to

obtain specimens, with which few reasonable people would argue, it ends by advocating some relaxation of local controls, on which it is difficult to comment without more knowledge of the local situation, and proposing a "Code of ethics for collectors and capturers of birds" with which few can disagree and doubtless most will warmly welcome. Since you were at one time very helpful with this campaign, and since the code surely deserves careful study by members of many disciplines throughout the world it may be useful to reprint it:

- The privileges of a collecting or capturing permit shall be used only to obtain specimens for justifiable scientific or educational purposes.

- Collect or capture specimens only from those populations or species that can sustain the loss of individuals.

- Collect or capture only those specimens that are deemed necessary and that can be properly cared for or prepared.

- Exercise the greatest care in recording accurately the maximum amount of relevant data for all specimens obtained.

- If live birds are collected, maintain them under humane conditions with high standards of health and sanitation.

- Collect with the aim of making available all relevant data obtained from specimens, either through publication or by giving access to the data.

- Abide by all stated regulations, including the use of authorised permits to collect, capture, import, export and trans-ship specimens.

- Notify the appropriate local authorities of plans to collect or capture birds in areas under their jurisdiction.

- Identify yourself and your purposes to those who may witness your collecting or capturing in order to inform them of the validity of your activities.

- Be as judicious and humane as possible in collecting and capturing activities, taking care to respect the rights, interests, and feelings of others.

- Regard the privilege to collect or capture birds as a trust in the pursuit of science; it should never be flaunted.

Yours faithfully,

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news and views

THE application of nuclear magnetic resonance (NMR) spectroscopy to biochemical systems is increasing at a prodigious rate, with the emphasis now clearly in the direction of enzyme-substrate interactions. As more and more sophisticated interpretations are brought to bear on indirect measurements, it becomes increasingly difficult for the people most interested in the biological implications of the experiments to follow the reasonings and assess the significance of the results. What, for example, does the fact that ion A is $x(\pm y)$ nm from atom B of substrate C bound to enzyme D tell us, in the absence of crystallographic data on the crystal structure? Those who have such reservations will surely welcome the trend exemplified by a research group from the National Institute of Medical Research in London. This group has been pursuing investigations into the enzyme dihydrofolate reductase, and its latest paper (page 564) concerns the direct observation by ^{31}P NMR spectroscopy of NADPH (a dinucleotide cofactor in the enzyme reaction) bound to this enzyme. The authors have been able to study some properties of the bound molecule directly, revealing some facts about the nature of the binding, and are clearly very close to being able to determine its conformation, well in advance of the determination of the enzyme structure by more conventional means.

Biological studies using ^{31}P NMR have been relatively few compared with those using proton NMR. This has been due largely to two factors. First, nearly all biological molecules contain hydrogen, but not all contain phosphorus.

NMR and enzyme binding

from G. E. Chapman

Second, the sensitivity of ^{31}P to NMR detection is about an order of magnitude less than that of protons, and hence correspondingly higher concentrations are needed. But ^{31}P has some distinct advantages over protons for NMR study. In particular the chemical shift range is greater, and linewidths are less than those of protons on the same molecules. This means that more fine structure may be observed in the ^{31}P NMR spectrum of a large molecule than in its proton NMR spectrum, and hence there is more potentially useful information to be extracted.

The earliest published papers in this branch of biological NMR dealt with nucleotides and related molecules, where the technique was used principally to investigate solution conformations in conjunction with proton NMR. Then work began to appear on phosphoproteins, nucleic acids and phospholipids, but it is probably fair to say that the triumph of observation outweighed any other scientific significance, at least in the earlier publications. A paper on ^{31}P NMR of tRNA fractions by Gueron (*FEBS Lett.*, **19**, 264; 1971) appeared to show the promise that conformational information could be derived from the chemical shift perturbations of the phosphodiester groups (in the same way that has been done for proteins by proton

NMR) but little has been heard since in this direction. The application of Fourier transform techniques to NMR caused a dramatic decrease in the concentrations of nuclei needed for observation. As a result of this, Richards and his coworkers at Oxford could achieve the remarkable feat of monitoring the changing levels of phosphorus-containing tissue metabolites in an intact muscle during tissue death, using ^{31}P NMR (*Nature*, **252**, 285; 1974). This highlights the potential value of NMR as a non-destructive assay technique. This type of experiment has been extended to the study of a developing embryo. There appears to be an additional bonus in that the chemical shift of the inorganic phosphate resonance makes possible an estimation of the intracellular pH without physiologically perturbing the system.

Evidence is accumulating that ^{31}P relaxation is dominated by chemical shift anisotropy at high magnetic fields. This apparently esoteric titbit is not without significance to prospective researchers in the field: in essence it means that the resolution of chemically different phosphorus nuclei in a sample, far from being increased by going to higher magnetic fields will probably be decreased. Thus there is much scope for work at the field strengths attained by conventional magnets, rather than using the much higher field (and costlier) superconducting magnets.

It is to be expected that ^{31}P NMR will play an increasing role in the study of biological systems at the molecular level. □

BRIGHT meteors often leave a faint, persistent luminosity along the visual path after they have passed. For most meteors this luminous train lasts for a very short time, but for some it may be observable for seconds, minutes or even an hour. This persistent train is not formed along the whole path of the glowing meteor nucleus but only along that part of the path which falls within a specific height region of the atmosphere, usually between 85 and 90 km above the Earth. The colour of the train is green or yellow, fading to white as the train decays, the observed spectrum consisting of very few bright lines. Trains illuminated by sunlight, however, are red or orange, mainly due to the reflection of sunlight from the

Persistent meteor trains

from David W. Hughes

fine dust particles left behind the ablating meteoroid. Trains expand laterally at a rate of about 1.6 m s^{-1} , so a train which persists for an hour is about 11 km across at the end of this time. Train luminosity, I , decays according to the formula $I = (a + bt)^{-2}$ where a and b are constants and t is the time.

Many meteor trains appear double after a time, the train gradually becoming a tube of luminous matter which,

viewed from the side, appears like a double line of light. This effect may be caused by the dying out of the luminosity along the train axis or by the expanding edge of the train having a greater luminosity. If the luminosity was distributed evenly throughout the cylindrical train it would appear brightest in the centre when viewed from the side. During their lifetime the trains are blown about by the prevailing winds in the mesopause region of the atmosphere and in the first quarter of the twentieth century meteor train movement was the only indicator of the winds and turbulence in the upper atmosphere.

Meteor showers differ strikingly in the relative numbers of persistent

meteor trains produced. For example, about 50% of the meteors in the Perseid shower (which maximise around August 13 each year) leave trains, while only about 5% of the Geminids do so (maximum rate on December 14). Plavec (*Bull. astr. Insts Csl.*, **2**, 19; 1949) concluded that the relative number of trains depended on the geocentric velocity of the meteoroids in the shower, this being 60 km s^{-1} for the Perseids as opposed to 35 km s^{-1} for the Geminids. Lindblad (*Meddn. Lunds astr. Obs.*, Ser. 1. No. 189; 1956) found that there was a direct linear relationship between the mean visual magnitude of the train and its duration, the brightest lasting longest. Also brighter meteors were more prone to have trains. Meteor magnitude, however, is not the only factor to be taken into account and often during shower observations bright meteors appearing in the same part of the sky and within a few seconds of each other differed radically in train-forming ability. Lindblad carried out radar and visual studies of the same meteor and found a clear tendency for radar echoes of exceptional duration to be associated with the presence of a visual train. In fact the train formation process is statistically more closely linked to the phenomena of a long duration echo than to that of a bright meteor—it is more dependent on the ionisation produced by the meteoroid than on the luminosity. In the magnitude range +2 to -1 the radar echo duration is twice as long for the train-forming meteor as for the non-train meteor.

The mechanism responsible for the persistent luminosity is still something of a mystery. In 1907 Trowbridge (*Astrophys. J.*, **26**, 95) suggested that gas phosphorescence was the cause and compared the phenomena with the afterglow produced in a gas by the electrodeless ring discharge. Chapman (*The Aurora and Airglow*, edit. by Armstrong, E.B., and Dalgarno, A., 204, Pergamon, 1955) thought it unlikely that the source of energy emitted over so long a period could be the meteor itself, and suggested that the luminous energy was drawn from a pre-existing store in the atmosphere itself, such as the energy of dissociation of oxygen. It is also necessary to explain why some but not all meteors from the same stream and of the same brightness produce trains. Chapman suggested that meteoroids contain some catalyst that can draw on the atmospheric store of energy and convert it into light and that this catalyst is only present in sufficient quantities in a certain percentage of the meteoroids. Sodium, which as the normal airglow shows can glow through the night, was suggested as the most probable candidate: it would

transform atomic oxygen to molecular oxygen by converting the dissociation energy of O_2 into sodium light.

Baggaley in this issue of *Nature* (page 567) produces quantitative calculations of the energetics of this sodium process. The complete sequence of necessary chemical reactions is known together with their relative rate coefficients. Also the concentration of sodium in the incident meteoroid is known with fair confidence because meteoroids have been found by spectroscopic analysis of the emitted light to have the same elemental composition as carbonaceous chondrite meteorites. The persistent trains seem to be confined to the 85–90 km height region of the atmosphere and rocket borne mass spectrometers have yielded the chemical concentrations of O, O_2 , O_3 and N_2 in this region.

Using all these observations Baggaley concludes that a meteor of magnitude about -10 (that is a tenth as bright as the full Moon) is required to introduce enough sodium to account for the observed luminosity. These very bright meteors are exceedingly rare—13 times rarer than the persistent meteor trains which last more than half an hour.

So it seems that the sodium process cannot provide sufficient luminosity and aeronomists must think again. Possibly the role of ionised species and the peculiar tube-like appearance of the train provide a clue. □

Information transfer in mammalian cells

from M. Bobrow and E. Solomon

Part of this article was inadvertently omitted last week. The earlier part described several situations in which genetic information, varying in amount from the whole nucleus to a small chromosomal fragment, can be transferred between different types of eukaryotic (and particularly mammalian) cells.

Transfer of genetic information by isolated mammalian DNA was claimed some time ago by Bendich, Borenfreund and Honda (*Informative Molecules in Biological Systems* (edit. by Ledoux), 81, North-Holland, 1971). Chinese hamster cells were incubated with mouse DNA and 1 in 10^5 – 10^6 cells subsequently appeared to react with an antiserum made to mouse cells. The alterations in these cells has not been further characterised. In a somewhat different situation, the technical difficulties in work of this sort are well demonstrated by the recent evidence (Kleinhofs *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2748; 1975) that bacterial

contamination was responsible for early claims of the integration of isolated DNA into plant chromosomes. There is always the possibility that misleading results can be given by bacterial, mycoplasma, or viral contamination of the material used.

The same group (Bendich, Borenfreund, and Sternberg, *Science*, **183**, 857; 1974) have recently investigated another potential system for transfer of genetic information. Mouse spermatozoa were coincubated with Chinese hamster fibroblasts and a proportion of these fibroblasts acquired the capacity to react with an antiserum to mouse embryos. Similar results have been obtained with rat sperm and Chinese hamster fibroblasts and were reported in *Nature* last week (Higgins *et al.*, *Nature*, **257**, 488; 1975). Clones of cells from these experiments react with an antiserum to rat embryo, but have no detectable rat chromosomes on karyotyping. This situation is therefore somewhat similar to the various experiments described earlier, in that a genetic function appears to be transferred in the absence of any specifically identifiable chromosome from the donor. The HPRT enzyme transferred by way of isolated metaphase chromosomes has, however, been well characterised by a variety of independent techniques as being of donor species origin, whereas the information transferred by coincubation with spermatozoa has thus far been defined only by immunofluorescence with a relatively crude antiserum to an undefined antigen in murine embryonal tissues.

Spermatozoa are a cell type evolved specifically to the function of cell fusion and the transfer of genetic information; and yet (although it is difficult to document from the published literature) many workers have tried without success to use spermatozoa in cell fusion experiments of various sorts. The specific interest in transferring genetic information from sperm as opposed to any other cell source is that if the technique could be sufficiently refined it would open the way to direct analysis of meiotic products, give access to a genome which has not yet been modified by the processes of tissue differentiation, and it could conceivably throw some light on the natural process of fertilisation. The possible production of embryonal antigens, and their relationship to malignant change is the particular interest of the group presently investigating this system.

The experimental transfer of eukaryotic genetic information by nuclear transplantation or virus-mediated cell fusion has already been proven of tremendous value in several fields of biological research. Whether a similarly bright future awaits the recently de-

scribed techniques for transferring smaller amounts of genetic information is difficult to predict. If, with the development of appropriate selective systems, it should turn out to be regularly possible to introduce bits of chromosome containing one or a few specific genes into host cells, this could turn out to be of major significance.

Year of the ψ

from W. T. Toner

The Lepton-Photon Symposium was held at Stanford University on August 21–27.

It is appropriate that this year's Lepton-Photon Symposium was at Stanford where many of the discoveries which have revitalised high energy physics have been made. Pride of place went to new results from electron-positron annihilation, many of them obtained at the host laboratory.

The spectroscopy of the ψ , as it stood before the symposium, was reviewed in *News and Views* for September 11. Two new states were added at the meeting, to make a total of at least eight. R. Schwitters (SLAC, Stanford) reported data from the SLAC-Berkeley collaboration showing a fourth ψ -like particle of mass 4,400 MeV and width 50 MeV, produced directly in electron-positron annihilation. B. Wiik (DESY, Hamburg) and J. Heintze (Heidelberg) both presented evidence for the decay chain $\psi(3095) \rightarrow \gamma X(2800) \rightarrow \gamma\gamma\gamma$, and Wiik tentatively suggested (on the basis of two events) that X may also decay to proton-antiproton pairs. Spins and parities remain to be determined except for the two lightest ψ s (1^-), but the uncanny resemblance of the level structure to that of positronium (Mills *et al.*, *Phys. Rev. Lett.*, **34**, 1541; 1975) left few to argue against some form of charmonium interpretation (Appelquist *et al.*, *Phys. Rev. Lett.*, **34**, 365; 1975; Eichten *et al.*, *ibid.*, 369).

If 1975 is the year of the ψ , 1976 may well be the year of the lepton. Earlier this year M. Perl (SLAC) had reported on 24 anomalous events of the type $e^+ + e^- \rightarrow \mu^\pm + e^\mp +$ unobserved particles, of which only 5 could be background from conventional processes. G. Feldman (SLAC) discussed the extension of this analysis to cover the whole of the SLAC-Berkeley data, leading to a total of 86 events, of which no more than 22–30 could be from conventional processes. It is possible that these events are due to the production and weak decay of a pair of charmed mesons, but the most

popular explanation (as yet without proof) is that the reaction is $e^+ + e^- \rightarrow U^+ + U^-$ where U is a heavy lepton, a member of the series $e, \mu, \dots$ and decays to $\mu\bar{\nu}_\mu\nu_U \rightarrow \nu_U$ is the neutrino associated with U. The excitation curve suggests a mass of about 1.9 GeV for U.

The most apt comment in the 36 years of our attempts to understand the leptons, is Rabi's "Whoever ordered that?", of the muon. With a third member of the series one might begin to make progress. F. Gilman (SLAC) remarked that the U (if a lepton) has been found where charmed mesons were expected, just as the muon was found where Yukawa's pion was sought. He left unspoken the outrageous thought which occurred to more than one member of the audience: that there might be a connection between the masses of these leptons and hadrons—the pion is not much heavier than the muon, and the charmed mesons (when found) should have masses ~ 2 GeV. H. Harari (Weizmann Institute) presented an imaginative scheme linking leptons to hadrons in another way: the electron to the u and d quarks which are found in ordinary matter, the muon to the s and c quarks to be found in strange and charmed particles respectively, and the U to new t and b quarks, which would make up other families of particles yet to be discovered.

L. Lederman (Columbia University) discussed old and new data on the direct production of the old leptons (muons and electrons) in the strong interactions of protons, a process that should not take place to any appreciable extent, but does (*News and Views*, March 13). The plot seems to be thickening, with reports of enhanced production, relative to pions, at small transverse momentum, which rules out any explanation in terms of large transverse momentum phenomena, and also makes it difficult to explain the effect in terms of any very heavy particle decaying into lepton pairs. There is experimental confusion at low energies, with new data from a Pennsylvania-Stony Brook collaboration showing direct lepton production at energies as low as 10 GeV, in agreement with Leipuner *et al.* (*Phys. Rev. Lett.*, **34**, 103; 1975), but in disagreement with Winter (*Phys. Lett.*, **B57**, 479; 1975) and with Russian data reported to the symposium. The new discoveries at the electron-positron storage rings do not seem to be related to the high-energy direct lepton production in any obvious way, and if direct leptons are indeed produced at low energy, a relation between the two is extremely unlikely. There is no explanation for this phenomenon, nor even any estimate of its likely significance. As his conclusion,

Lederman showed a blank transparency.

The symposium would still have been stimulating without all this excitement. There was, for example, the strong evidence presented by Schwitters for the production of back-to-back jets of particles in electron-positron annihilation at 7.8 GeV, in beautiful agreement with the parton model; there was the report by W. Lee (Columbia University) of the first clear observation of the long sought decay of the ρ' (1600) into two pions; and a great deal more. There was also the 'monopole' event (Price *et al.*, *Phys. Rev. Lett.*, **35**, 487; 1975), which L. Alvarez (Berkeley) argued, most convincingly, was probably caused by the fragmentation of a platinum nucleus in the detector.

There was such euphoria about the new physics that several theorists—Gilman, Harari, C. H. Llewellyn-Smith (Oxford) and J. D. Bjorken (SLAC)—went out of their way to remind us that much of the interpretation rests on plausible assumptions, which remain to be proved. This will require a searching re-examination of the old physics as well as the new. $\square$

More headaches from star formation

from M. G. Edmunds

A RENEWED discussion of the interpretation of observational studies of the radio spectra of molecules in the Orion nebula will probably make star formation theorists reach for their aspirin bottles. The theory of star formation is complex and difficult; the refinements of competing physical processes that can be invoked are almost infinite, and yet even the most simplified models gobble up large amounts of computing time, only to leave the proponents arguing about numerical errors.

The basic model attempts to follow the self-gravitational collapse of a cloud of interstellar medium into a state where it is hot and dense enough for the start of nuclear energy generation and the birth of a star. Any observational constraints on the theories are invaluable since the conditions involved must range over many orders of magnitude in temperature and density as the formation proceeds. The presence of large amounts of finely-divided dust in the dense gas clouds which are the site of star formation means that radiation at optical wavelengths is obscured and the probing of the physical conditions can only be achieved with radio and infrared techniques. One of the happiest hunting grounds for the elusive observational data has been the region of the Orion nebula, which includes both the optically visible region of gas heated

by young hot stars, and a nearby extended region of high infrared luminosity believed to represent a nursery of stars in the process of formation.

Zuckerman and Palmer (*Astrophys. J. Lett.*, **199**, L35; 1975) consider the molecular lines in the radio spectra of the Orion infrared complex. They point out that the lines show a composite spectrum of a sharp spike superimposed on a broad plateau. The widths of the lines may be attributed to broadening by the Doppler effect as a result of gas motions; the spike implying velocities of about 4 km s^{-1} and the plateau implying much larger velocities of perhaps 30 km s^{-1} . While the low velocity region extends over a large region, the high velocity region appears much smaller and is centred on an area where the infrared emission is very high, including the sources known as the Becklin-Neugebauer object and the Kleinmann-Low infrared nebula. It therefore seems that the gas in a small region of Orion experiences much greater motions than the rest of the nebula. The most obvious interpretation is an increased velocity as a result of a conservation of energy and angular momentum during the collapse of part of the gas to form a protostar. But it is puzzling that inorganic molecules such as SiO, SO, H₂S are far more prominent in the high velocity region (compared with molecules containing carbon) than in the low velocity region. Since the

conditions required to excite the lines of these inorganic molecules are very similar to those required for the organic compounds, the implication is that the molecular composition of the gas in the Orion nebula is not homogeneous. Zuckerman and Palmer speculate that the elemental composition of the gas may not be homogeneous, and if this were the case then the consequences for the chemical composition of the forming stars would be fundamental. A less extreme view is that the conditions for molecule formation differ between the kinematically distinct regions, while the elemental abundances remain constant.

There is an alternative interpretation of the observations. Zuckerman and Palmer recall the suggestion that the Becklin-Neugebauer object represents an evolved F star whose radiation is very reddened by dust (Penston, Allen and Hyland, *Astrophys. J. Lett.*, **170**, L33; 1971). Indeed, the only other "plateau" molecular line source yet found is a carbon star whose molecular spectrum shows organic molecules formed from outflowing gas rich in carbon synthesised by nuclear reactions during the star's evolution. The Orion plateau source could therefore be explained as a region of gas ejected from a normal evolved oxygen-rich star in which molecules formed from oxygen dominate over those formed from carbon. There have been objections to the interpretation of the very strong

Becklin-Neugebauer object as a reddened evolved source, but if the above argument is correct there is justification for the view that at least some of the infrared sources in the Orion complex represent evolved stars rather than natal protostars.

The more depressing news is that Zuckerman and Palmer snatch away from theorists some observational determinations of the magnetic field in regions of star formation. They suggest that the existence of high velocity broadening effectively vitiates a recent determination of a very strong magnetic field by the Zeeman-effect broadening of SiO lines. They also point out that observational selection effects in OH line Zeeman-effect determinations, and uncertainties over what density of region these maser lines originate in, exclude such measurements as a guide to the general magnetic field within the nebula. They finally suggest that the measurement of polarisation of infrared emission induced by the alignment of grains in a magnetic field will not even give the field to an accuracy better than a few orders of magnitude. All this is very frustrating to model makers since, in a slightly ionised gas cloud, the magnetic field acts as an effective pressure and may be considerably enhanced as the cloud collapses. The strength of the field is thus an important parameter in determining the dynamics of the collapse. □

HALF the elements of the first transition series, namely manganese, iron, cobalt, copper and zinc have biochemical roles which are well documented and, in the main, thoroughly investigated. Of the remaining five elements of the series the investigation of three is increasing in popularity. Since 1911 (Henze, M., *Z. physiol. Chem.*, **72**, 494) vanadium has been known to occur in high concentration in the blood cells of ascidians—sea squirts—although neither its function nor its chemistry is yet understood. More recently both chromium and nickel have come under scrutiny for possible biological roles.

Chromium is now known to have an important function in nutrition in both man and animals, being involved in glucose metabolism (Merz, W., *Physiol. Rev.*, **49**, 163; 1969). Dietary studies have shown that induced chromium deficiency in rats and monkeys leads to impaired glucose tolerance, giving rise to a diabetes-like syndrome. This observation naturally led to studies of the effect of chromium on the action of insulin. Enhancement of the response of adipose tissue and mitochondria to insulin was observed with many chromium (III) species although

Biochemistry of transition metals

from A. J. Thomson

non-labile complexes such as $[\text{Cr(III)}(\text{ethylenediamine})_3]^{3+}$ were ineffective. In view of the recent detailed structural studies of insulin by Hodgkin and co-workers (*Proc. R. Soc.*, **B186**, 192; 1972) this problem invites further examination.

Nickel stood out among its transition metal neighbours as apparently being without biological effect. Sunderman and his colleagues have long sought one, concentrating at the outset on the toxicity of nickel carbonyl (*Ann. N.Y. Acad. Sci.*, **199**, 300; 1972). They have now demonstrated that nickel is maintained in the blood sera of man and experimental animals at levels between 2 and $12 \mu\text{g l}^{-1}$. The element is especially abundant in rabbit serum, facilitating isolation of a protein containing nickel, termed nickeloplasmin, which immunoelectrophoresis identified as an α_2 -macroglobulin. The protein has a high molecular weight ($>7 \times 10^5$) and contains 0.9 g atoms of

nickel per mol of protein. Now a group of Australian workers (Dixon, N. E., Gazzola, C., Blakeley, R. L., and Zerner, B., *J. Am. chem. Soc.*, **97**, 413; 1975) have advanced the claim that jack bean urease is a metallo-enzyme containing nickel. Although it was the first enzyme to be crystallised by Sumner in 1920 the metal content had escaped detection. This group has now succeeded because they had available a large quantity of highly purified enzyme. Atomic absorption and gravimetric analysis gives an estimate of 2.0 ± 0.3 g atoms of nickel per 105,000 g of enzyme. The electronic spectrum has been recorded and confirms the presence of the d-d transitions of the nickel (II) ion. Since the extinction coefficients of octahedral nickel (II) d-d transitions are very small, usually of the order of $10 \text{ mol}^{-1} \text{ cm}^2$, the detection of these bands requires long path lengths of highly concentrated material. For example, to detect the spectrum of nickel (II) in carboxypeptidase A, in which the naturally occurring zinc ion had been replaced by nickel, it was necessary to use 50-mm path length cells and $\sim 5 \times 10^{-4} \text{ M}$ protein to yield absorbance values of only ~ 0.05 (Rosenberg, R. C., Root, C. A., and

Gray, H. B., *J. Am. chem. Soc.*, **91**, 21; 1975). It is presumably partly for this reason that the nickel ion in urease has remained hidden for so long.

Vanadium is present in high concentrations in the blood cells of certain tunicates, marine animals which feed by filtering seawater. The metal is contained within clusters of vacuoles, called the vanadophores, each about 2 μm in diameter, which are themselves housed in the blood cells or vanadocytes, 8 μm in diameter. The vanadophores, which are pale green, are known to contain vanadium (III) ions and 1.8 N sulphuric acid. But the exact nature of the vanadium ions within the vanadophore is not clear. The difficulty arises because of the fragility of the vacuoles and because of their instability towards oxygen. The vacuoles lyse readily in distilled water to give a dark red-brown haemolysate with a pH of 2.5. Known as Henze solution it is presumed to contain vanadium in the trivalent state. When neutralised the solution turns deep blue, possibly by oxidation to form the vanadyl ion, VO^{2+} . In Henze solution the vanadium is bound to a protein termed homovanadin and to a small organic nitrogenous ligand (Bielig, H. J., *et al.*, *Protides Biol. Fluids*, **14**, 197; 1966). It was hypothesised that in the intact vanadophores the vanadium is likewise bound to a protein and small ligand. Now Carlson (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2217; 1975) has re-examined the problem using proton NMR spectroscopy to probe the vanadium ion within intact vanadophores of the tunicate *Ascidia ceratodes*. Since lysing of the vacuoles offers the opportunity for oxidation, denaturation and artefact formation in the haemolysate it is an advantage to be able to probe the metal ion *in situ*.

The NMR spectrum of whole blood cells exhibits a broad 21-p.p.m. downfield, Gaussian signal which disappears on cell lysis. By comparison of this signal with the sharper signal, also at 21 p.p.m., found in 0.72 molal solution of vanadium (III) chloride in 0.5 M perchloric acid the signal is attributed to vanadophore water protons in rapid exchange with the protons of the water in the co-ordination sphere of vanadium inside the vacuoles. Coupled with the intensity of the signal the broadness argues against the signal being due to protons of a stable vanadium (III) complex. Carlson further points out that as the 21-p.p.m. signal decreases in amplitude and shifts upfield on lysis simple dilution is not occurring but rather oxidation and/or complex formation with a high molecular weight material. He also attempts a quantitative estimate of the vanadium (III) molal concentration by comparing the bulk resonance shifts of water in the spectra

of suitable vanadium (III) standard solutions with the magnitude of the 21.5-p.p.m. signal. This leads to a figure for the amount of vanadium accounted for by the 21-p.p.m. signal and, knowing the total vanadium content from atomic absorption studies, the maximum number of vanadophore vanadium (III) co-ordination sites interacting with ligands other than water *in vivo*. The conclusion is reached that a maximum of one co-ordination site per vanadium (III) ion is left available for binding an organic ligand. This view of a partially complexed vanadium (III) ion within the vanadophore is not in complete agreement with the views advanced earlier by Bielig.

Reviewing the function of the vanadium vacuole in the light of his recent work, Carlson proposes that the vanadocytes have properties not shown by the haemolysate, the system usually studied. It may even be necessary to regard the vacuole as the smallest functional unit. Carlisle (*Proc. R. Soc.*, **B171**, 31; 1968) has shown that intact tunicate blood cells act as oxygen storage and transport units although the blood haemolysate does not oxygenate reversibly. Carlson's work suggests a way out of this apparent contradiction. Further probe studies of the intact vanadocytes will be of great interest. Measurement of the electron spin resonance spectrum and the electronic spectrum using microspectrophotometry are two obvious techniques.

Beyond this, ascidians should have an increasing fascination for chemists in their search for biological functions of metals beyond the first transition series. In his 1968 review, Carlisle reports that many other metal ions besides vanadium are actively accumulated by different species of marine animals, for example, "titanium, chromium, manganese, iron, niobium, and tantalum, certainly, and possibly also molybdenum, zirconium, and tungsten. In all the animals investigated the metals are found in blood cells . . . associated with acidic conditions in which they exhibit a powerful reducing action." It is well known that at low pH the early transition metal ions will condense to form polymeric oxy-anion clusters but only in their higher oxidation states. Goldberg (*Mem. geol. Soc. Am.*, **67** (Treatise, 1), 345; 1957) has shown that these metals are actively accumulated since a lifetime of passive filtration could not pass sufficient water through ascidians to yield the levels which have been reported. The enrichment factors he quotes are spectacular. For example, for marine organisms by dry weight over seawater, titanium is enriched 10,000-fold, vanadium 280,000-fold and niobium 98,000-fold. Very little of the chemistry of these

processes is understood although in future the technological and environmental importance of such scavenging of metal ions will undoubtedly increase.

Hill reaction in the mountains

from Peter D. Moore

WHEN Turesson began his classic work on development of ecologically adapted races within species (*Hereditas*, **3**, 211; 1922), he paved the way for what has proved to be a profitable interaction between genetics, ecology and, more recently, biochemistry. In the early days of such studies, genetic strains could be detected only by structural variation, but recently it has become possible to document more subtle, physiological adaptations to differing environmental circumstances within certain species. Particular attention has been paid to temperature optima of selected biochemical systems from species at different altitudes or latitudes.

A system that has been investigated in several species is the Hill reaction of photosynthesis. This process, which is observed in isolated chloroplasts, when illuminated, involves the movement of electrons from water to an added chemical oxidant (such as potassium ferricyanide) against the chemical potential gradient. The reaction involves the production of oxygen, a process which is thereby demonstrated to be separable from carbon dioxide reduction in photosynthesis.

In 1968, Tieszen and Helgager (*Nature*, **219**, 1066) described experiments with populations of the grass *Deschampsia caespitosa* from arctic (Alaska) and alpine (Colorado) habitats. They measured the response of the Hill reaction (as ferricyanide reduction) to a range of temperatures and observed that the optimum for reaction was approximately 10 °C higher in the alpine population than in the arctic one. This appears to be related to the environmental conditions in the two habitats, for the alpine site would have higher daytime temperatures, whereas survival in the arctic conditions would be favoured by maintained activity even at low temperature. As with the work of Mooney and Billings on *Oxyria digyna* (*Ecol. Monogr.*, **31**, 1; 1961) a degree of flexibility was found if growth conditions were varied, particularly in the alpine population.

May and Villarreal (*Photosynthetica*, **8**, 73; 1974) subsequently examined the Hill reaction in populations of *Taraxacum officinale* from three different alti-

tudes in the Rocky Mountains of Colorado. When assayed at 15 ° and 25 °C populations from high altitudes (3,550 m) had the greatest rate of Hill activity. The reverse was true at 35 °C when the material from the highest site was virtually inactive. The authors suggest that the high rate of the Hill reaction in the elevated sites is an adaptation for fast growth during the short alpine season. This is coupled with a lower temperature optimum at the alpine site. This presupposes that the selection pressure for rapid growth is greater at high altitude than at low altitude. This is conceivable since other pressures, perhaps involving seed production and competition during establishment, may be more significant at lower altitudes. It would be interesting to couple these biochemical investigations with studies of the population biology of these plants in the vein of Gadgil and Solbrig's work on *Taraxacum* (*Am. Nat.*, **106**, 14; 1972). It would be of value to know whether the populations examined had been subjected to predominantly r and/or K selection within their respective habitats, for this could determine the relative survival value of rapid growth.

May and Villarreal collected their material from the field and analysed it within 4 h. They were not able, therefore, to conduct experiments in which growth conditions were varied and in which phenotypic acclimation could be separated from genotypic adaptation. In addition, the apomictic breeding system of *Taraxacum* makes it difficult to know how many separate genotypes are involved in these experiments. It has been demonstrated, however, that even in out-breeding species, such as *Abies balsamea*, the temperature optimum for growth varies considerably along altitudinal gradient (Fryer and Ledig, *Can. J. Bot.*, **50**, 1231; 1972).

It would be unwise to extrapolate from these data to make predictions for other species, however, as the results of Williams, Lazor and Yourgrau (*Photosynthetica*, **9**, 35; 1975) demonstrate. They have taken samples of *Verbascum thapsus* from several latitudes (30°, 47° and 51°) in the United States and also at several altitudes within the same latitude (in Colorado, of course). In contrast to the results of previous workers, they found that the high altitude populations had lower rates of Hill activity and there was no clear cut difference in temperature optima. In their latitudinal studies they found that low latitude populations showed higher activity than those of high latitudes. Thus the negative relationship between Hill activity and length of growing season shown by May and Villarreal for *Taraxacum*, does not hold for *Verbascum*. The very broad range of temperature over which *Verbascum* is

capable of maintaining high rates of Hill reaction is a notable feature of these studies. This could contribute to the success of this species as a weed of disturbed habitats in North America as well as in Europe and many other parts of the world.

Two main points emerge from these pieces of work. First, differences in Hill reaction rate and differences in the temperature optima for Hill activity exist between isolated plant populations, and there is evidence suggesting that these differences are genetically based. Second, it has become apparent that further advances in the understanding of these processes can only be achieved by making use of controlled environment growth experimentation. This is needed to differentiate between genetic and phenotypic adaptation within test populations and to document changes which may occur in the rate of Hill reaction with season or with advancing physiological age of the tissue being tested. □

Large scale shell model calculations

from N. MacDonald

A meeting on Large Scale Shell Model Calculations was held in Glasgow on August 27-29.

THE shell model plays a major role in the understanding of nuclear structure. Most of the particles in the nucleus are treated as inert, while the remaining few active particles are, to a lowest approximation, treated as moving independently in a common central potential. This approximation does not get one very far, and one calls on an effective interaction between pairs of active particles.

Two essential questions are thus raised. One is how to devise an effective interaction that can be related to the known features of interactions between free nucleons. The other is how many of the particles are to be treated as active, and how many states are to be regarded as accessible to them. About three years ago a substantial advance was made in handling moderately large numbers of particles. The technical problem involved is that of diagonalising very large matrices. R. Whitehead recognised that the key to this lay in the revival of the Lanczos algorithm, an iterative method involving repeated multiplication of an arbitrary vector by the matrix. When the vector has the form of a shell model wavefunction and the matrix

is that representing the two-body effective interaction, this iteration can be performed without ever storing the elements of the matrix.

The Lanczos method is the essential feature of the Glasgow shell model code, which has been used for very complete surveys of the nuclei from mass 17 to 39, over which the active particles fill the s-d shell. The meeting was held to review the progress made in these surveys, and the status of the shell model generally.

B. Barrett (University of Arizona, Tucson) reviewed the theory of the effective interaction. The problem is to go from a known interaction and a large set of states to an interaction yielding a good approximation when restricted to a much smaller set. The method used is perturbation expansion. Convergence is controlled by the extent to which states of the excluded part of the large set overlap in energy with those of the small set. For the s-d nuclei these intruder states undoubtedly lie at embarrassingly low energy. More sophisticated ways (double partition) of dividing up the large set of states may yet yield an effective interaction with a sound foundation, but at present shell model calculations are best tackled in a less ambitious spirit.

This means starting from a subset of the available data on a range of nuclei and determining the interaction that fits this best. Then one can test it on the remaining data. This process can start from a simple form of interaction, as in work on the s-d shell reported by P. W. M. Glaudemans (University of Utrecht) and other Dutch workers, who start from a surface delta interaction. They vary seven parameters to provide a preliminary estimate, and then carry out an iterative variation process on all 63 matrix elements. One may instead elect to vary all the two-body matrix elements without any constraints. The most ambitious attempt of this kind was reported by B. H. Wildenthal (Michigan State University). He used the Glasgow code in an unsuccessful attempt to locate such a best fitting interaction to data from the whole shell. Failing this, he was able to provide separate interactions for the upper and lower halves of the shell. These supersede the earlier Michigan interactions found by fitting data over smaller sets of nuclei with the Oak Ridge codes.

The next step into larger spaces, as for example nuclei of mass 40 to 70, goes beyond our present powers of computation. It becomes essential to truncate the space used. It was hoped that with the Glasgow and Michigan results for the s-d shell one might be able to devise a trustworthy truncation scheme. One approach reported by

P. Manakos (University of Darmstadt) and J. Millener (Oxford University) uses the SU3 scheme, which relies on the approximate validity of certain symmetries in nuclear states. Another (J. Morrison, University of Glasgow) is to employ a deformed rather than a spherical common potential. A danger which emerged in studies of truncation, in particular in those reported by A. Watt (University of Glasgow), is that the best strategy may depend very much on the interaction used. But in any higher shell the interaction may be quite different from those used in the s-d shell. A consolation is that in studying this question one learns quite a lot about the nature of the states ground out by the mammoth computations.

The most mysterious feature of the Lanczos method in its nuclear applications is the extremely rapid convergence of the iterations. Almost independently of the size of matrix, 50 to 100 steps suffice to get the desired low states. R. Whitehead (University of Glasgow) presented various results related to this. One is the formal relationship, by way of the technique of Pade approximants, between the Lanczos method and the inverse iteration method. Another is that performing 50 multiplications by the energy matrix, starting with an arbitrary vector, is related to evaluating the first 50 moments of the energy in an arbitrary state. Techniques for studying low moments, which were discussed by O. Bohigas (Orsay), could conceivably be extended to approach the shell model in a different way. □

European earth scientists seek closer collaboration

from A. J. Smith

The meeting of European Geological Societies (MEGS) organised jointly by the Geological Society of London and the University of Reading was held at Reading from September 8 to 12.

THE theme of the meeting, which was attended by about 400 earth scientists, was 'Europe from Crust to Core', and early announcements expressed the hope that geologists and geophysicists "would discuss the crucial scientific and socio-economic questions affecting European geology in the next 100 years". The programme promised that the theme and ideal might be achieved and in the event, thanks to excellent organisation and a warming by those

present to the concept of a collective European image, the promoters can feel well satisfied. In their opening remarks Sir Kingsley Dunham (Institute of Geological Sciences, London) and P. Allen (University of Reading) urged participants to cross political and disciplinary boundaries for the benefit of geological science, Europe and the rest of the world, a theme to which J. M. Harrison (UNESCO, Paris) returned on the closing day of the meeting.

The programme was built upon Stille's concept of Europe's crustal framework, with thirteen keynote addresses, numerous shorter contributions and ample time for formal and informal discussion. The opening keynote address by V. E. Khain (Moscow State University) was concerned with the new tectonic map of Europe which is to appear in 1977. He reviewed the wealth of geological information which has become available since the production of the first tectonic map of Europe less than a decade ago. Both he and Janet Watson (Imperial College, London), in her address on the evolution of the European craton, drew attention to the strong influence of a Precambrian framework on the succeeding development of the European crust. Major structural lines, once started, have reasserted themselves and influenced succeeding events. Other keynote speakers and contributors made clear that ocean floor spreading did not offer a panacea for Europe's lithospheric history and, instead, that gravity tectonics, often along old lines, frequently offer a more satisfactory explanation. A. Kvale (University of Bergen) pleaded for more work to increase the scientific value of plate tectonic models applied to the European Caledonides, while W. Krebs (Braunschweig Technical University) questioned the existence of any Palaeozoic 'oceanic' crust anywhere in central Europe, preferring the concept of repeated metamorphism and plutonism caused by ascending mantle diapirs. H. Ramberg (Uppsala University), Krebs and many others saw the driving force of folding, thrusting, allochthonous gliding and nappe transport as resulting from the potential relief energy between rising zones and synchronously subsiding adjacent troughs. Sir Peter Kent (Natural Environment Research Council), dealing with new knowledge from the North Sea, drew attention to the relationship between freer movement of plates in North-western Europe from mid-Cretaceous times consequent on the parting of the crust in the Atlantic replacing the stretching of marginal continental crust. He also drew attention to the need for a reappraisal of accepted theories for the origin of the Permian evaporites. A. Aubouin (Université

Pierre et Marie Curie, Paris), in his summary of the Alpine orogeny of the eastern Mediterranean, while accepting the 'classical' plate tectonic approach, though without the need for entrapped microplates in the eastern Mediterranean, stressed the need for more work to explain the numerous enigmas. E. Niggli (University of Berne) instanced the problem of the depth of crystallisation of blue schists and the possibility of tectonic stripping of cover.

Geophysical topics concerned with the deepest crust, mantle and core naturally ranged beyond the limits of the European continent. Several geophysicists drew attention to the contrasting crustal thickness between adjacent European areas—the non-sedimentary crusts beneath Cainozoic depressions being some 30 to 45% less than beneath the Hercynian massifs.

Many speakers stressed the role of the geologist as a forecaster as well as a recorder of events. G. F. Mitchell (Trinity College, Dublin) made much of this in his Quaternary review, as did M. Arnould (Ecole National Supérieure, Paris) and G. Luttig (Geological Survey, Hanover, FDR). Geothermal energy, the disposal of radioactive wastes, environmental and conservation topics were discussed in the European context. Speakers both in the main conference and at the meet-



A hundred years ago

IT is rather disappointing that Capt. Young's Arctic Expedition in the *Pandora*, which arrived at Portsmouth on Saturday, should have returned home prematurely without accomplishing any part of the work for which it was organised—the discovery of additional Franklin relics and the complete navigation of the North-west Passage. Under the circumstances, however, Capt. Young has adopted the wisest possible course. Better that the expedition should spend a comfortable winter at home, and set out early next year to renew the attempt in which they have just been baffled. Disco was reached on August 7, Upernivik on the 13th, and Cape York on the 16th, after a splendid passage through the much dreaded Melville Bay. Carey Islands were visited to deposit letters for the *Alert* and *Discovery* and to obtain a despatch from Capt. Nares, as previously agreed on. The despatch, however, was not discovered till the return voyage.

From *Nature*, 12, 539, October 21, 1875.

ing of the Commission on the Tectonics of Ore Deposits, which met concurrently with MEGS, described the value of ERTS (Earth resources technology satellite) imagery in a greater understanding of Europe's structure and in the search for mineral resources. Pleas that geologists generally should pay more attention to the special needs of engineers and planners were common, and the need for more mathematical, chemical and experimental approaches to earth science was examined.

European geologists were reminded of their obligation to the rest of the world in more than just an economic sense: G. Jenkins (University of Canterbury, Christchurch, New Zealand) reminded them that stratotypes, the key to the worldwide geological division of time, were often first described in Europe, and that to aid worldwide

correlation more must be researched in Europe and published.

A perhaps oversimplified view of the meeting might be that extremely useful summaries of the state of European geology were given, showing that Europe still presents fundamental questions in need of answers. Seeking such answers can occupy European geologists, geochemists and geophysicists for a long time to come. Time, particularly in respect to resources, will not wait for geologists any more than for the rest of mankind, and the participants recognised that closer collaboration between European earth scientists is urgently needed.

About a year ago correspondence in *Nature* referred to MEGS under the heading 'European earth scientists dis-unite', suggesting that a schism had opened between European 'geologists' and geophysicists. In this context it is

a pity that so few geophysicists attended MEGS for they would have enhanced the meeting. The Reading meeting showed that European earth scientists sincerely desire to act in concert for the benefit of Europe and the world. The UK initiative which created MEGS was warmly appreciated. The establishment of a European Geological Society was discussed and received wide support but no final decision was reached. Instead it was decided to set up an interim committee composed of representatives of a number of the participating countries to promote a MEGS 2 (representatives of both the Netherlands and France indicated a desire to host such a meeting), to cooperate with existing international and European bodies and to encourage closer communication between European earth science societies. □

CONTRARY to widespread belief, although the steady state theory of the Universe may be dead it refuses to lie down, and a small band of enthusiasts continues to try to breathe life into the presumed corpse. But the chances of a miraculous resuscitation do not look too good, as the appearance of two papers together in the same issue of *Mon. Not. R. astr. Soc.* shows. In one of these contributions (172, 623; 1975) P. K. Das of the Tata Institute develops further the model of QSOs as compact, highly gravitationally redshifted objects that he and J. V. Narlikar put forward recently (*Mon. Not. R. astr. Soc.* 171, 87; 1975); although Das does not mention the steady state theory, it seems a reasonable assumption that any colleague of Narlikar's who discusses possible non-cosmological contributions to the redshifts of QSOs has the concept not too far towards the back of his mind.

This model has a great deal of interest for theorists and students of relativity, whatever its application in the real Universe, and consists of a static, spherically symmetric distribution of a mass M in a radius R (Das uses dimensional units), in hydrostatic equilibrium, obeying Einstein's equations and divided into two regions, core and envelope. The earlier paper from Das and Narlikar developed equations for the isothermal core-adiabatic stable envelope configuration and discussed some implications for redshifts; in his new work Das concentrates on a discussion of how light propagates from both the surface and interior of such an object, and the effect of this on the angular diameter seen by an external observer.

The observed features of such an object depend on whether R is greater

One step forward and two back for the steady state

from John Gribbin

than or less than $3M$, and the ratio R/M is reduced by 'stiffening' either the core or envelope, that is, making the model more relativistic. For light emitted from the surface, as the core or envelope equation of state is stiffened the angular diameter decreases from its Euclidean value; for sufficiently stiff core-envelope combinations, the back of the source becomes visible, with the entire surface eventually being mapped into a thin ring.

But the source need not appear as a ring, and light emitted from the interior can play a part in determining the overall appearance. Combining the effects on light from the core and from the surface with the earlier investigation of redshifts, Das is able to produce an angular diameter-redshift ($\theta(z)$) relationship. He finds that as the core-envelope combination is stiffened a plot of θ against z shows a minimum, which shifts to higher z as the stiffening increases, and "it is interesting to note that θ calculated on the basis of cosmological redshift hypotheses also shows a minimum at comparable values of z ".

The properties of this model do depend critically on the equation of state, and for pressure as high as half the energy density the angular dia-

meter is constant irrespective of surface area, with multiple imaging taking place. Of course, radiation from the interior will be absorbed by intermediate material *en route* to the observer, and investigation of the expected absorption feature is the logical next step in developing this intriguing model. Intriguing though it is, however, it is a little artificial, and its relevance to the real world must be in even greater doubt in view of the paper by V. K. Kapahi (ironically, also of the Tata Institute) in the same issue of *Mon. Not. R. astr. Soc.* (172, 513; 1975). He has looked at angular size counts of extragalactic radio sources, using the 3CR catalogue, and finds evidence of evolution in source properties even when QSOs are excluded from the sample. This is already difficult to reconcile with steady state ideas, and the same sort of evolution also seems to be present when QSOs are analysed separately, which suggests rather strongly that QSOs really are part of the same family as other extragalactic radio sources.

The best explanation of these variations is to fit them to a simple Einstein-de Sitter cosmology in which the local radio luminosity function (RLF) steepens considerably at high luminosities, the comoving density of high luminosity sources increases with z in a manner similar to that implied by number-luminosity ($\log N - \log S$) studies and the V/V_m test for QSOs, and mean physical sizes of radio sources evolve with z approximately as $(1+z)^{-1}$. This important confirmation of the pattern suggested by the earlier studies hints strongly that even if a way can be found of mimicking QSO properties in other ways, the cosmological interpretation stands better than ever before. □

Computer supplement

How Babbage's dream came true

M. V. Wilkes

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Charles Babbage dreamed up a form of computer in the 1830s. His ideas lay dormant until digital machines became a reality in the 1940s. Professor Wilkes, one of those responsible for the implementation of the digital computer, describes some of the prehistory and early history.

COMPUTERS have a history and a prehistory. If we confine ourselves to electronic computers the first historical date in the computer era is February 18, 1946 when the ENIAC was formally inaugurated at the Moore School of Electrical Engineering in the University of Pennsylvania. Nine months earlier, in May 1945, the Automatic Sequence Controlled Calculator had been set going at Harvard University. It was not an electronic machine but was based on the use of rotating shafts, electromagnetic clutches, relays, and so on. It had, however, the distinction of being the first fully automatic digital computer to be completed. Its origins can be traced back to a report written by Howard H. Aiken in 1937. The machine was built by International Business Machines (IBM) with Aiken's vigorous co-operation. A number of machines using telephone relays for computing purposes were built. As early as 1938 there had been interest in this subject at the Bell Telephone Laboratories and a number of special purpose devices had been built and used. This work, however, did not culminate in the construction of a completely automatic calculating machine until 1946. Zuse also worked on relay computing devices in Germany during the latter part of the war. Randell¹ provides documentation of this period.

The prehistory of the subject formed the life's work of just one man, Charles Babbage (1792–1871). Although he never completed his machine Babbage had all the ideas and L. J. Comrie when writing for *Nature* a review of a book describing the Automatic Sequence Controlled Calculator had the happy thought to entitle it "Babbage's Dream Comes True". I have adapted this title for the present article.

Babbage began to think about calculating machines in about 1812 or 1813 at a time when he was still a student at Cambridge. His interest had been caught by a very ambitious project for computing mathematical tables started in France during the time of the first Republic. The principle adopted in this project was to compute accurate values at rather widely spaced values of the argument, and to produce values at the required tabular interval by systematic interpolation or subtabulation. Babbage saw that this could be done by a machine which he called the Difference Engine. Not only would this have calculated tabular values, but it would also have prepared the moulds from which stereo plates for printing could be cast. This was an important point and Babbage thoroughly appreciated the importance of avoiding copying and typesetting errors.

Babbage became very enthusiastic about the Difference Engine and successfully communicated his enthusiasm to the

British Government, who gave him financial support. All went well to begin with and he spent a number of years happily engaged in supervising the construction of the engine. Unfortunately, however, a high rate of progress was not maintained and difficulties arose between Babbage and his engineer. Eventually the project had to be abandoned without there being anything to show for it.

Babbage's mind was, however, working on generalisations of the Difference Engine and suddenly around 1834, when he was 42 years old, he began to perceive how a truly general purpose calculating machine could be made. One idea followed another in rapid succession. On September 18, 1834, he wrote to his close friend John Herschel, the astronomer, who was then working at the Cape of Good Hope: "I have begun to criticise all I have done and to consider it merely as the bricks of a better fabric. In the first place I have simplified and can, therefore, again generalise. . . . In short I see my way on towards turning the whole of analysis into wheel work as far, at least, as numerical results go, and this will at last come"². The intellectual excitement that Babbage was experiencing at that time is well brought out in an earlier letter to Herschel: "I suppose like myself you have never one hour out of the twenty-four to spare; yet I should like, if possible, to catch one to discuss with you some of the *stray* questions about which I cannot resist indulging myself as a refreshment from the Analytical Engine"³. He goes on to mention several topics, such as the theory and practice of cutting tools, how to make a real automaton, and so on.

The machine that Babbage named the Analytical Engine was—or would have been had it been built—a true, automatic digital computer. Like a modern computer it was to have a store in which numbers could be held, and a processor known as the "mill" in which the arithmetic operations would be performed. For the control of the calculations, Babbage at first proposed to use a drum with moveable studs, but almost at once he took the important step of deciding to adopt the Jacquard mechanism that had then come into general use for controlling looms. His ideas developed very rapidly and by the end of 1837 he had drafted an account setting them out in some detail. For the rest of his life Babbage worked on the design of the Analytical Engine, improving and refining his ideas, but his account of 1837 contains all the essentials (see ref. 1).

For some reason or other Babbage did not publish this account; in fact there is very little in his published writings about the detailed way in which his ideas would be implemented. He described the functions of the store and the mill, but he did not even introduce a term to describe what we would now call the control unit. A good deal of information was, however, published about the general principles on which the Analytical Engine worked and the type of calculation for which it could be used. In 1843 Babbage gave a series of presentations in Turin. An account of these was written by Menabrea and published in a Swiss journal. The paper was translated from

the original French by Lady Lovelace who added extensive notes, written under Babbage's close supervision. The idea of repetitive loops within a calculation was explained, but the actual way in which they were set up was not described. Lady Lovelace gives a number of what appear to be programs, but close examination shows that only the arithmetic operations are described; the repetitions are indicated by comments or by statements in the text that certain series of operations are to be repeated so many times. Babbage proposed to use separate Jacquard mechanisms to control operations and variables; these would step independently and go through independent cycles. For some reason he never arrived at the idea, so familiar to us, of an instruction consisting of an operation code associated permanently with one or more addresses. It is, however, clear from his notebooks and other unpublished writings that he gave very detailed thought to the sequencing mechanism⁴. Associated with each Jacquard mechanism he proposed to have a "card counting apparatus" or "repeating apparatus". This

would be loaded with an integer which would be decremented whenever a card was read. When the counter liquidated ('running up' was Babbage's term) an appropriate action would ensue. Babbage is not anywhere fully explicit about how the system would work and I doubt if he ever entirely made up his mind between the various alternatives. He proposed to systematise the internal control by making use of "barrels", or drums with projecting studs, a system that offers a striking parallel to microprogramming.

Reading Babbage makes one realise what an important advance was the development of the modern concept of a program as something which can exist as a valid statement of an algorithm independently of the computer on which it will run and about which things may even be proved. Part of the breakthrough of the stored program computer was the development of a language—initially machine language only—in which programs could be written. If Babbage had described in formal terms the role played by his card counting apparatus and had introduced a notation for the way in which the Jacquard cards were punched, he might have taken this vital step; however, the fact that he had independently stepping Jacquard mechanisms to control operations and variables would have made the programs difficult to follow, and they would not have borne much resemblance to modern programs.

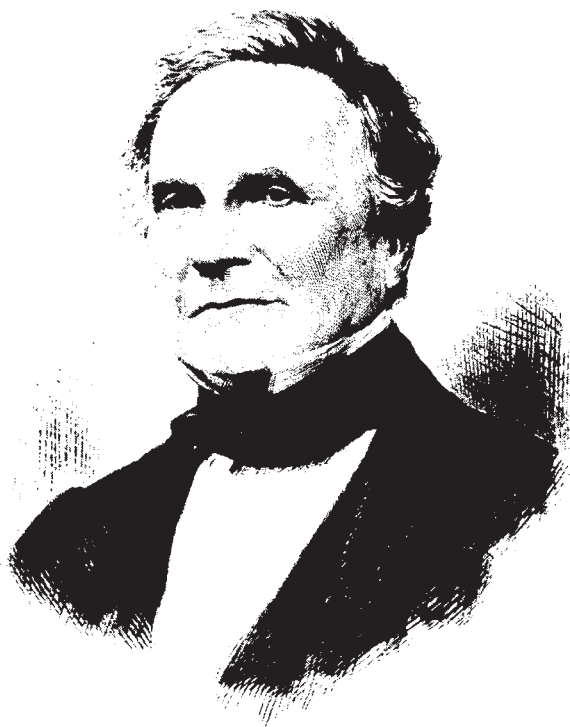
One must ask why, in the end, Babbage had so little to show by way of practical achievement. I think that he might well have hoped to finish the Difference Engine. The ideas were sound enough, as was shown by the fact that another implementation of them, by George and Edward Sheutz, was successful; however, that was a good deal later, in 1855, and the techniques of mechanical engineering were developing rapidly at that period. Much of the responsibility for the failure of the Difference Engine must fall on Joseph Clement, whom Babbage employed to be in charge of the construction. In April 1828 Herschel, who was looking after things during Babbage's temporary absence in Italy, clearly thought that the progress being made was inadequate in view of the amount of money being spent. At that time Clement was employing 10 people on the project⁵. Babbage, however, continued to have full confidence in Clement and it was not until five years later that they finally fell out.

The Analytical Engine was another matter. I doubt if it would have been possible for Babbage to have produced a full scale machine that would work at all reliably. To do so using the mechanical principles he had in mind would be far from easy today. In view of what had happened to the Difference Engine, there was little prospect of his getting the necessary financial support to set up a proper project. Babbage was, however, prepared to spend his own money and always had people in his employ; after his death his younger son carried on the work for some time. As a result many drawings were made and some pieces of the Analytical Engine were constructed; these are now to be seen in the Science Museum at South Kensington.

Babbage's real achievements were intellectual, and they remained buried in his notebooks. In March 1839 he did indeed draft a plan of a work entitled *The Science of Number Reduced to Mechanism*⁶. He revived this project at various times and it is hard to see why he did not persevere with it, particularly since he was an accomplished writer and published extensively on other subjects. If he had done so he might well have shown the way for someone else to put his ideas into practice. As it was they were lost and what was of value in them had to be re-discovered.

We must, I think, conclude that, although a mathematician and a man of science, Babbage was, as far as calculating machines are concerned, an inventor at heart. That is to say he saw achievement in terms of the successful construction of a device and not in terms of the publication of ideas. Inventors are vulnerable to the hazards of the world and as likely as not end by becoming bitter and disappointed men. Babbage had more than a twinge of such feelings, but fortunately the Analytical Engine, although it occupied a large part of his life, was not his sole preoccupation. His other interests were wide and varied

Charles Babbage (1792–1871)



Let it be granted that in his life there was much to cause disappointment, and that the results of his labours, however great, are below his powers. Can we withhold our tribute of admiration to one who throughout his long life inflexibly devoted his exertions to the most lofty subjects? Some will cultivate science as an amusement, others as a source of pecuniary profit, or the means of gaining popularity. Mr. Babbage was one of those whose genius urged them against everything conducive to their immediate interests. He nobly upheld the character of a discoverer and inventor, despising any less reward than to carry out the highest conception which his mind brought forth. His very failures arose from no want of industry or ability, but from excess of resolution that his aims should be at the very highest. In these money-making days can we forget that he expended almost a fortune on his task? If, as people think, wealth and luxury are corrupting society, should they omit to honour one of whom it may be truly said, in the words of Merlin, that the single wish of his heart was "to give them greater minds"?

From W. S. Jevons's obituary notice of Charles Babbage (*Nature*, 5, 28–29; November 9, 1871).

and he led an active social life. He could be tiresome and self-important, but he had many friends and was by no means lacking in humanity, as anyone reading his autobiography will appreciate.

When I became interested in computing in the years immediately before the Second World War, L. J. Comrie and others were showing great ingenuity in using commercial accounting machines for scientific purposes and they took great delight in exploiting their special features in new ways. The climate of opinion was, however, distinctly hostile to the production of special digital machines for scientific calculation, either by the adaptation of commercially available machines or otherwise. On the other hand, various analogue devices were coming along; these included the differential analyser, announced by Bush in 1931, and various linear equation solvers. The scientific world was prepared to give these a sympathetic reception and it suffered the inevitable disappointment. In fact, when the EDSAC began to work in Cambridge, one of my tasks was to persuade people that it was not like another analogue device with its own esoteric techniques, but that it handled proper numbers in the proper way. Fortunately, I was helped by the fact that the second program run on the EDSAC was one for the listing of prime numbers, something manifestly respectable from an arithmetic point of view.

The EDSAC began to work in May 1949 and other early computers came into operation at about the same time. These were the first stored program computers—the ENIAC had been programmed by means of a system of plugs and sockets and switches—and the development of the new subject of programming then became possible. As might be expected, different groups followed their individual ways and there was a good deal of diversity. That was not only because the computers had their own instruction sets but also arose for more or less accidental reasons connected with the background and attitudes of the staffs involved. Computer peripherals were in a primitive state of development and the capabilities of a computer were much coloured by what it happened to possess in this respect. This was particularly true because all the early computers had very small high speed memories. The Pilot ACE at the National Physical Laboratory (NPL), for example, used punched cards for input and output, and it was possible to punch 12 binary numbers on a card, one on each row. Punched cards could thus be used as an auxiliary memory and the machine was very suitable for performing matrix calculations. The NPL, therefore, took a lead in that aspect of computing, with consequences that remain to this day. In Cambridge we had punched paper tape for input and output, and a mechanical read-only memory from which a set of initial orders could be copied into the high speed memory to control the reading of the program tape. We were, therefore, led to develop a format for instructions that may be regarded as the forerunner of later assembly languages. At Manchester, they had a magnetic drum as well as a high speed memory from the very beginning, and they therefore developed methods of programming that exploited a large auxiliary memory earlier than the rest of us. This diversity was very helpful to the development of the subject, and we all learnt much from comparing notes. Those who were active in the small computer world of the late 1940s and the 1950s will remember the regular colloquia held in Cambridge on certain Thursday afternoons, which provided a good opportunity for the experts to compare information. They also served the purpose of those who wished to inform themselves about what was going on at a time when published material was scarce and contact with one or more of the established centres was essential.

It came as a surprise to me personally to find that it was so difficult to get programs right. In Cambridge there was much emphasis both on programming aids to debugging and on methods of constructing programs that would be relatively fool-proof. The use of previously tested subroutines was one such method, and we recommended the construction of a program by means of nested, closed subroutines with program para-

meters; if one substitutes the words 'procedure call' for 'closed subroutine', we have here one of the central tenets of structured programming. A consequence of the adoption of these methods was that we found little need to draw flow diagrams, and I have always felt that the motto on the structured programming banner should be "flow diagrams considered harmful".

Along with the development of programming techniques went the development of computer applications. An early problem to be solved on the EDSAC was one proposed by R. A. Fisher⁷. He wrote to me early in 1949, before the EDSAC was working, saying in characteristically barbed fashion that he understood that our fine new machine was not going to be used only for wave mechanics and that he would like to have a numerical solution of a certain second order nonlinear differential equation with two point boundary conditions. I do not think that he had much hope that we would produce the solution, but one was, in fact, obtained by D. J. Wheeler as part of his thesis work. Fisher was duly impressed and he eventually published the solution with a suitable acknowledgement.

Atomic calculations were, of course, an obvious field of application for an electronic computer, although we realised that the early machines would be somewhat lacking in capacity. I remember one day accidentally running into S. F. Boys at Imperial College. We had known each other in Cambridge before the war and he asked me what I was doing now. I told him about the EDSAC under construction and rather to my surprise he began to explain to me why such a machine would not be of any use for the type of atomic calculation that he was interested in. I kept my counsel, but later, when Boys had returned to Cambridge and became an enthusiastic user of the EDSAC, he was only too ready to confess how wrong he had been. The point that he had failed to grasp was that a digital computer could perform logical as well as arithmetic operations; the EDSAC had one order for performing a logical operation, namely, that of bit by bit Boolean multiplication of two binary numbers. This was known as the 'collate' order and Boys found that it made all the difference.

I was more than willing to tell people about the EDSAC, but I never made any attempt to sell them the idea of using it if I met with sales resistance. To do so was unnecessary since there was no shortage of users. At first, professional programmers did not exist and people had either to learn to do their own programming or persuade someone who was working with a machine to do it for them. Our own research students were the best ambassadors. Computer applications in X-ray crystallography were pioneered in Cambridge by J. C. Kendrew, then a junior member of the staff of the Cavendish Laboratory, and J. M. Bennett, a research student with us. This led in due course to the calculations for the structure of myoglobin that were done on EDSAC 2. It was through contacts of this kind that the use of a digital computer spread in Cambridge, and I am quite sure that the same is true of other places.

In the very early days it was possible for the management of a computer centre to follow in some detail what all the users were doing. We had in the laboratory a body known as the Priorities Committee before which all applicants for computer time would appear to explain their problem. In spite of its name, the Priorities Committee was essentially a technical committee, and the members would discuss with the applicant the best way to go about his work, the best numerical procedures to use, and so on. From time to time, the applicant would come back to tell the committee how he was getting on. I found these regular meetings of great interest, since they brought me into touch with a wide and ever expanding variety of subjects, as more and more research workers realised that a computer could be of assistance to them. In the course of time, much of the work of the committee became routine, but it still continued to discuss the more interesting projects.

I have mentioned that one of the very early programs to be run on the EDSAC was one for listing prime numbers. This particular program worked by the crude method of dividing each odd number in turn by all the odd numbers not greater

than the square root. The division was done by repeated subtraction since the EDSAC had no divider. It took about 10 minutes to compute and print the first 170 primes. Wheeler later wrote a program that was essentially a mechanisation of the sieve of Eratosthenes. The EDSAC had a cathode ray tube monitor on which the contents of the high speed memory could be displayed in raster form. It was possible to show 16 35-bit words at a time, that is, 560 bits altogether. In Wheeler's program the odd numbers were represented in order by binary digits. To begin with, all these digits were ones, indicating that all the numbers were present. As the sieve operated and numbers were eliminated the ones were replaced by zeroes. The speed of the machine was such that it was possible to watch this happening on the screen.

J. C. P. Miller, who joined the Laboratory at the beginning of 1950, had a long-standing interest in prime numbers and he and Wheeler set about seeing whether they could break the record for the largest known prime number. This was the Mersenne number, $P = 2^{127} - 1$, which had, some 75 years before, been shown by Edouard Lucas to be prime. Miller and Wheeler prepared a routine for testing the primality of numbers of the form $kP + 1$ and soon found ten values of k that gave prime numbers. On June 7, 1951 they wrote a letter to *Nature* reporting this fact⁸ and giving the largest known prime number as $934P + 1$. They added a note in proof (October 8) giving two even larger primes, the larger of which, $180P^2 + 1$, was the largest then known. They added that A. Ferrier, using a desk machine, had just demonstrated the primality of another large number, and that this was the second largest known prime. Though we were glad that the EDSAC had broken the record, we had much sympathy with Ferrier, who after great labour with a desk machine, had had the trophy snatched from his hands at the last moment by the march of technology. In another way we were fortunate. The prize, by all justice, should have gone to Manchester University, where somewhat earlier a baby model computer had been demonstrated. This was very limited in that it had no reader for punched cards or paper tape,

and no printer, and it could only perform the operation of subtraction; moreover, its memory was minute. Nevertheless, with great skill, a program for testing Mersenne numbers for primality was written, and quite a lot of numbers were tested. Alas, none of them were primes.

Ryle has related in his Nobel address⁹ how the development of radiotelescopes working on the principle of aperture synthesis depended on the availability of adequate computing power. I had a particular interest in anything to do with radio, since my own graduate work had been in the field of radio wave propagation and had been done at the Cavendish Laboratory. Pilot experiments in aperture synthesis were done with EDSAC 1 and EDSAC 2 and showed that a more powerful machine would be needed to handle routine reductions for a large telescope. Fortunately, such a machine was planned to come into operation in 1963 and I was glad to assure Ryle that the time would be available. When the telescope came into operation, there was a standing instruction in the laboratory that the routine reductions for it should have priority over all other work. In the ordinary way, this was not important, since the amount of time required was not great, but it did mean that, when the service was disrupted by maintenance or for other reasons, the telescope came first. It was one of Ryle's colleagues working on optical astronomy who made the remark in a lecture that the telescope was no longer the most important instrument used in astronomy, having been displaced by the computer. That was a clear exaggeration, but it showed what had been achieved in the space of a few years.

¹ *The Origins of Digital Computers: Selected Papers* (edit. by Randell, B.), (Springer, Berlin, 1973).

² Royal Society, London, HS 2 287.

³ Royal Society, London, HS 2 305.

⁴ Wilkes, M. V., *Babbage Memorial Meeting* (British Computer Society, London, 1971).

⁵ Royal Society, London, HS 2 225.

⁶ *Buxton Papers*, Museum of the History of Science, Oxford.

⁷ Fisher, R. A., *Biometrics*, 6, 353 (1951).

⁸ Miller, J. C. P., and Wheeler, D. J., *Nature*, 158, 838 (1951).

⁹ Ryle, M., *Les Prix Nobel en 1974* (Stockholm, in the press).

Whatever happened to the Stantec Zebra?

David Davies

University central computing needs in Britain are provided for by an increasingly unified set of machines. The next step is likely to involve more comprehensive networking.

BRITISH universities, with about 250,000 students, 40,000 of these at the graduate level, have roughly £58 million of capital equipment on their premises in the form of central computers, and the annual expenditure on hardware, buildings and recurrent costs runs at about £12 million.

Central policy on large computers is the concern of the Computer Board for Universities and Research Councils, which recommends action to the Department of Education and Science on university computing facilities; the board, under the chairmanship of Dr A. H. Chilver (Cranfield Institute of Technology) comprises eight members. It further gives advice to the research councils, although it has no control over their choice. It also reviews longer term policy on computers, which means these days that it does a fair bit of persuading universities that they will have to live with what they have

got for a year or two longer than expected. In the most recent report from the board (in August) broad hints were being dropped that university computers should have a lifetime of 10 years or more, "longer . . . than has been usual in the past". The problem the board faces is that much of its money is committed years in advance and any governmental squeeze is therefore difficult to apply in an even-handed way.

Time was when the university computer laboratory was a place where universities learnt how to build computers and research workers learnt how to operate them. Less than 15 years ago I can recall working in a computer laboratory where the staff used to leave a pile of chasses on the bench each night when they went home. If the machine went wrong, you ran a special program which produced a high pitched note through a loudspeaker. You took a mallet, banged the chasses one after the other, and when the pitch changed you knew you'd found the dud one, which you then replaced. The staff topped up the valves on the dud chassis in the morning.

All that has, alas, gone and in retrospect the turning point at which quirky originality had to be sacrificed to reliable uniformity was the publication in 1966 of the Report of the

Table 1 Recommendations of the Flowers report

University	Had in 1965	Flowers recommended	Has now
Cardiff	Stantec Zebra	Elliott 503	ICL 4/70
Sheffield	Mercury	ICT 1907	1907 and 1906S
Belfast	Deuce (borrowed from US Navy)	ICT 1905	ICL 1906S
Keele	(Private property of Professor McWeeny)	ICT 1903	ICL 4130
Leicester	Elliott 803	Add an Elliott 503	Cyber 72
Aberystwyth	IBM 1620	Elliott 4100	Two ICL 4130s

Flowers Working Group on Computers for Research. The group, charged with assessing university and research council needs for five years, found a wide diversity of incompatible machinery, most of it inadequate either in size or reliability for the burgeoning demands for computer time. The report correctly noted that many scientists were desperate for computer time—"Swansea will shortly be accessible from Aberystwyth only by private car, and that requires 2½ hours driving over mountain roads", "one man [at a London college] found it quicker to compute in Canada, flying there and back at frequent intervals", and so on. Remote terminals were only just coming in; Project MAC with an IBM 7094 at MIT was described admiringly.

Flowers urged that three very large computers be installed in London, Manchester and Edinburgh, which would serve as regional centres. Individual universities should have better computing facilities and compatibility with the regional computers. "The system would thus form an integrated whole." Some of the recommendations of the report are shown in Table 1. Expenditure was expected to be £20.5 million for universities over a period of five years.

The "very large machines" for the regional centres would have to be American. There was an interesting discussion in the report of the problems of keeping up with IBM, but it was clear that the IBM 360/92 and the CDC 6800 were the only real competitors.

The government accepted almost entirely the generous and detailed suggestions of the Flowers report; because of the "economic situation" it would take six years instead of five to implement the recommendations. The three regional centres were set up and a fourth, well equipped centre aimed at providing a focus for the universities in the south-west (Bath, Bristol, Cardiff, Exeter and the University of Wales Institute of Science and Technology—UWIST) is in process of being established at Bath (see Table 2). In addition other universities have been virtually re-equipped since 1966. A summary of computing equipment installed or ordered for all universities is given in Table 3. The Computer Board says, a touch laconically, "without any undue complacency, we feel the aims set out in Flowers Report have, to a large extent, been achieved".

What Flowers could not foresee, and what has added to the importance of the regional centres, has been the emergence of remote terminals as a way of life. Most universities are either on a network such as the South-West network, or possess Computer Technology Limited 'Satellite One's which for £20,000 put the universities in touch, by phone line, with a

regional centre. No more 2½ hour drives through the Welsh mountains; even the tiny and remote St David's, Lampeter now calls up Swansea on its Satellite One.

The health of British university computing is very much intermingled with the health of ICL; three-fifths of the capital investment is in ICL machines. The procedure for purchasing computers runs roughly as follows. For computers of power greater than one Atlas (the Atlas was a computer built in the 1960s; powers are measured by complicated assessments using benchmark programs) ICL may be invited to submit, singly, a tender. If, however, it is obvious that someone else can offer a cheaper and better machine over a shorter time scale the purchaser can go to open tender, but one of these tenders has to be from a British Company (which will be ICL). At the very large end (20 Atlas-power) ICL does not compete with the CDC 7600. But at 10 or so Atlas-power, the ICL 2980 in the 2900 range becomes competitive.

When ICL launched its new 2900 range in October 1974 it already had £21 million of orders, of which British universities had contributed £5 million. Ironically, when (shortly thereafter) the European Space Research Organisation (ESRO) ordered two 2980s they could only be supplied by persuading the universities to hold off for six months so that ICL could honour the ESRO contract.

Is the character of computing changing? In the past the regional centres were conceived as number-crunching establishments where immense calculations could simmer for hours on end. The number of scientists crunching numbers is not rising dramatically although, given half a chance, some of those who have contented themselves with one- and two-dimensional calculations would add an extra dimension. They are not likely to get that chance as the demand which is growing and which Flowers perceived in 1966 is for data manipulation. In such work the requirements are much more for easy accessibility of data bases, be they reference bases such as census results or dynamic bases such as meteorological conditions. There is little point in doing such operations down a telephone line, since the amount of data-shipping to interactive facilities may be colossal; the local centre comes very much into its own.

Ideally such data bases reside at the local computer, since they

Table 3 Equipment installed or on order at March 31, 1975

Manufacturer	Series (No. of machines)	Approximate capital investment (£k)
ICL	KDF9 (2) [formerly English Electric]	490
	1900 (29) [formerly ICT]	21,140
	4100 (11) [formerly Elliott]	2,480
	System 4 (10) [formerly English Electric]	6,080
	2900 (3)	5,640
	Total	35,830
IBM	System 360 (3)	2,130
	System 370 (3)	5,570
	Total	7,700
CDC	6000 (3)	4,680
	7000 (2)	4,460
	Cyber 72 (3)	1,580
	Total	10,720
DEC	PDP10 (1)	540
Burroughs	B1700/B5500/B6700 (3)	669
CTL	Satellite One/Modular One (39)	1,800

Table 2 Equipment at Regional Centres

Centre	Equipment
Edinburgh	2 × ICL 4/75s; IBM 370/158; ICL 2980 (on order)
London	CDC 7600/6600/6400; Cyber 72
Manchester	ICL 1906A; CDC 7600; Cyber 72
Bath (not officially a Regional Centre)	2 × ICL 4/50s; ICL 2980 (on order)

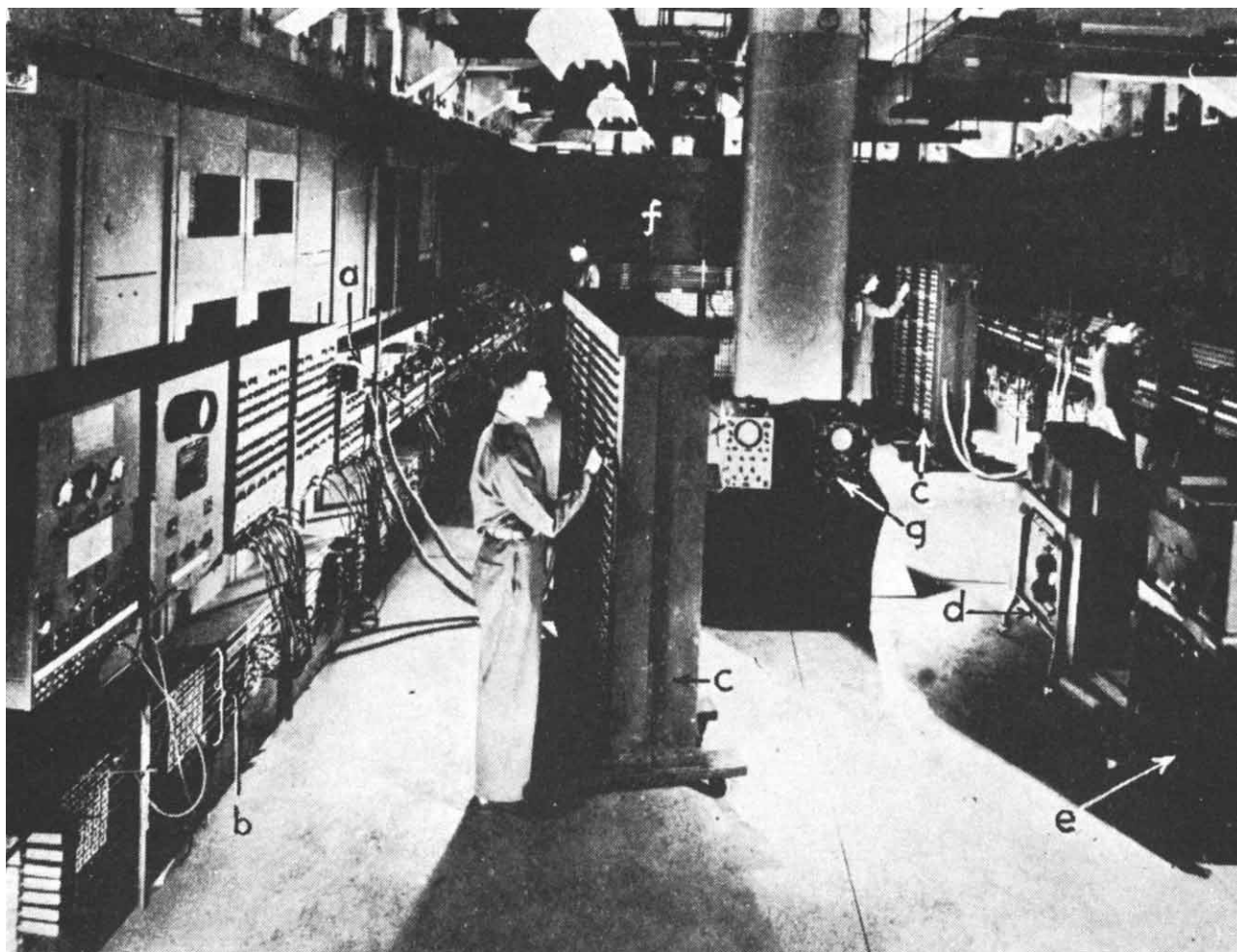
cost a lot to ship, but networking of a more comprehensive kind (such as Professor Kirstein describes in this supplement) is gradually becoming a serious alternative. The use of a network to distribute computing load is already explicit in the present phone-in arrangements to regional centres. The use of a network to handle a data base at a distance, or even a distributed data base in several parts of the country, requires further refinements; a scientist in Exeter must be able to handle a data base in Aberdeen by using programs resident in Aberdeen or even in East Anglia.

To this end several universities have expressed interest in using the Post Office's Experimental Packet Switching Service (EPSS) shortly to start operating and permitting data to be transferred at up to 48 kbits per second on a shared and sorted basis rather than by means of a continuous link. The eyes of the

more ardent data processors are bound also to turn towards the European Informatics Network, a project under the auspices of COST, the European Programme of Collaboration in Science and Technology. Ten nations are signatories to the project and at present five centres are to be joined by a packet switched network; the British link is through the National Physical Laboratory, Teddington. The network was not designed specifically for university use although universities in Milan and Zurich will be on it but it is possible that in the future it could be accessed through the EPSS to Teddington.

As far as bigger machines are concerned, there is little on the horizon. Sophisticated processors such as ILLIAC 4 or CDC Star 100 are not being thought of in a university context either in Britain or Europe, at least within the next five years.

The ENIAC, an electronic calculating machine



a, Digit trays; b, program trays; c, function unit; d, card reader; e, card punch; f, high speed multiplier; g, testing equipment.

The machine is built up in the form of a number of units each consisting of one or more vertical panels about 8 ft. high and 2 ft. wide, of which there are altogether forty. Each panel carries, at the back, racks of valves, relays, etc., and, at the front, switches, indicating lamps, plugs, sockets, etc. The different units are interconnected by two sets of lines, one set carrying signals expressing numerical information and the other set for control signals; connexions to and from these sets of lines can be plugged into the various units.

The whole machine comprises about 18,000 electronic valves, 3,000 indicating lamps, and 5,000 switches, and takes about 150 kW. in operation. Its flexibility and speed of operation will make it possible to carry out many numerical calculations, in many fields of investigation, which without its assistance would have been regarded as much too long and laborious to undertake.

Taken from D. R. Hartree's report in *Nature* (157, 527; April 20, 1946). See also *Nature*, 158, 500-506; October 12, 1946.

The credibility of machine intelligence

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We usually use our previous experience in solving a present problem, and 'intelligence' is the name of the mechanism by which we do this. A programmed computer should not be deemed intelligent unless it is functionally similar to the brain in its use of previous experience.

WHEN Christopher Columbus sailed westwards in 1492 he hoped to reach India, and that is why the indigenous native Americans are called Indians. In 1975 there is an undercurrent of opinion that, in its established usage, the term artificial intelligence (AI) is as much a misnomer as Indian is a misnomer for the indigenous American. If AI is misnamed this is surely due to the difficulties of making a clear comparison between the problem-solving faculties of the brain and a computer.

To understand how the brain works, in the way that we know how an ordinary digital computer works, is perhaps one of the most important and urgent technical problems of the present time. The solution could provide scope for advanced automation that would very substantially alter and perhaps improve the present commercial and industrial scene. A mechanistic understanding of thought processes might to some extent benefit psychiatry. If you consult a heart specialist you can be assured that he knows fairly accurately how the heart works, but a man who consults a psychiatrist has no such assurance concerning the brain.

Science usually progresses by testing hypotheses or models. A computer that has been programmed to play chess, prove theorems, or understand natural language, may be claimed to model a thought process. To make scientific progress we should eventually compare such programs with natural thought processes (more completely than in protocol analysis).

Comparing computer circuitry and algorithms

In patent specifications of digital data processing apparatus the detailed physical implementation of 'and' gates and other common components is usually not specified. Instead, these components are usually specified merely as 'and' gates, 'or' gates and so on, and logical design diagrams are given which show how the various components are interconnected. For the purposes of patents, it may not matter whether the logical design of computer circuitry is implemented by transistor integrated circuits or by relay switching circuits. Thus, two computers may be deemed to be embodiments of the same invention even though, so to speak, their detailed physiology is very different.

By means of Boolean algebraic manipulation, a logical design can be changed to an equivalent logical design that looks superficially different. For instance a two-input 'and' gate can be replaced by a two-input 'or' gate that has its two inputs and its output inverted. Thus, if the logical design diagrams of two computer circuits are different, this does not necessarily mean that there is really an essential difference between them. Even if the logical designs were essentially somewhat different, this difference might be judged, for example in a legal action over a patent, to be so

slight that the two circuits should be deemed to be embodiments of the same invention.

It may be more difficult to make such judgments on algorithms that are more abstract and complicated than circuits usually are. Two algorithms are said to be structurally equivalent if, and only if, for any given set of input values the algorithms execute the same sequence of statements and follow the same sequence of branches². There are generally many essentially similar but structurally non-equivalent algorithms for computing any given function, just as there are equivalent alternative logical designs for the hardware that implements any given switching function.

Two algorithms are said to be functionally equivalent if, and only if, for any given set of input values they yield the same output values. For purposes such as proving correctness of algorithms, structural equivalence seems too tight a notion, functional equivalence seems too loose a notion, and some sort of compromise between structural and functional equivalence is required².

Comparing brains with computers

In 1975 there is considerable knowledge of the physiology of nerve cells and the neuroanatomy of, for instance, cat, octopus, and man, but this does not provide us with a credible and generally accepted logical design for the brain or even for a part such as the visual system. We could, however, determine structural equivalence of two algorithms without using knowledge of any circuit diagram of any computer that could execute the algorithms, and absence of a circuit diagram of a brain should not therefore make us abandon hope of designing a machine that is in some useful sense equivalent to a brain.

A man may move a hand, or perform some other act, not in response to an external stimulus, but for some internal reason. A man may perceive an external stimulus but not take any physical action. Unlike many algorithms, therefore, a brain is not merely a device that determines an output corresponding to a given input, and this presents a difficulty in the determination of functional equivalence. In view of this we might confine our attention to the subset of behaviour that consists of making observable responses to stimuli, and determine whether there is functional equivalence between a brain and a model at least over this subset. There would be so many possible stimuli that we could not try them all in turn, and instead we might merely test that for a randomly chosen subset of stimuli the brain and the model made the same response.

Unfortunately this test would not establish functional equivalence, because it ignores the vital fact that a man's response to a stimulus generally does not depend on that stimulus alone, but also on his previous experience of stimuli and responses, and possibly on further rational and emotional factors. In other words, a man's output at any time may depend not only on his present input, but also on many past inputs and outputs. To be deemed functionally equivalent to a brain, a model should exhibit the same dependence of an output on many past inputs and outputs. This is particularly important if the model is intended to be intelligent, because natural intelligence is

concerned with using stored data derived from previous inputs and outputs to determine an output corresponding to a newly given input³. AI should surely model natural intelligence in this important respect, but the practical difficulties of determining this functional equivalence seem to be hopelessly great, because it is necessary to consider sets of previous stimulus-response pairs, and there are too many possible choices of the sets.

Nevertheless, it may be useful to pursue this discussion of functional equivalence a little further. A man's response to a stimulus may depend on previous experience, but not uniquely. That is to say, there are generally countless different histories of previous experience that will yield the same stimulus-response behaviour in a given situation. Conversely, a man may respond differently to the same stimulus on different occasions, without any significant change in his experience in the intervening time. In spite of the lack of a 1:1 correspondence between previous history and present stimulus-response behaviour, this behaviour generally depends on the application of intelligence to previous experience. To understand intelligence we should surely understand this dependence, and not confine our attention to present stimulus-response behaviour.

The solving of a problem, for example, proving a theorem, should for the purposes of this discussion be regarded as present stimulus-response behaviour. We usually use our previous experience in solving a present problem, and intelligence is the mechanism by which we do this. This is true even though there are usually countless different episodes of past experience that would serve us just as well. Intelligence is surely the mechanism by which we arrive at a procedure for solving a problem: intelligence is not the procedure itself. A model would be functionally equivalent to a brain only if the model used the same inputs of past experience as the brain and did not use any inputs that the brain did not use.

If a man copied from a text book a proof of a geometry theorem, we would not say in common parlance that he had used his intelligence in proving the theorem. If a man proved a theorem by executing a given theorem-proving algorithm manually, we would not say that he had used his intelligence in proving the theorem, because we would not be satisfied that he had intelligently made use of his own past experience. If a machine proves a theorem by executing an algorithm written by a man, this does not necessarily mean that the machine is intelligent, because from the vital point of view of inputs of past experience, the man and machine may not be functionally equivalent theorem provers, and indeed they may be radically and drastically different theorem provers.

Even if the functional equivalence of past experience to a brain could be determined, it would not provide a satisfactory criterion for intelligence of a machine, because a brain has other things going on in it (instinct for example) besides intelligence. Furthermore, intelligence has many different levels and different manifestations⁴. It is possible that a whole class of algorithms, not just one, should properly be called intelligent. Many somewhat different algorithms are properly classified as top-down parsing algorithms. Many somewhat different algorithms are properly classified as linear programming algorithms. To be classified as a linear programming algorithm, an algorithm need not be functionally equivalent to any given linear programming algorithm. An algorithm might be properly classified as intelligent even if it were not functionally equivalent to any given intelligent algorithm or to any brain.

Exact functional equivalence seems too strong a criterion, so we must fall back on a looser notion of functional similarity. It would surely be wrong to say that a model was intelligent unless it was functionally similar to

an intelligent brain in its use of previous experience in problem solving.

Not comparing brains with computers

McCarthy says that the main scientific activity of AI researchers is, or should be, "studying the structure of information and the structure of problem solving processes independently of its realisation in animals or humans"⁵. McCarthy's statement broadly defines an area within the field of computer science, and if only some name other than artificial intelligence had been assigned to this area, there would be less confusion and misunderstanding. Just as one is not free to define an artificial diamond to be a lump of coal, so also one is not free to assign the name 'artificial intelligence' to a field of study that may only be obscurely related to intelligence. All such relationships are likely to remain obscure until intelligence is precisely, scientifically, and acceptably defined.

But by clarifying judiciously chosen computational problems, and by discovering and comparing different methods by which these problems can be solved, we may perhaps eventually begin to agree that some problem solving processes are more intelligent than others. It is possible that two solutions to a given problem may be equally successful from a pragmatic point of view, but one solution may be judged more intelligent than the other in view of the type of past-experience data that it uses.

The use of computers in weather forecasting and in the computation of rocket trajectories involves machine representation of knowledge but does not belong to the AI field, presumably because the computations are numerical. AI^{6,7} is concerned primarily with those non-numerical problems, such as chess, theorem proving, and robot control, at which men excel over computers. In solving such problems there are two schools of thought in AI, the generalist and the specialist⁸. The specialist school is prepared to make more use of *ad hoc* programming than the generalist school.

To make a computer exhibit limited understanding of natural language^{9,10} it is convenient for the programmer to use his knowledge of the world, that is, his semantic knowledge, in writing procedures, and this practice is called procedural embedding of knowledge. This practice is characteristic of the specialist school⁸. When new knowledge is to be used, new *ad hoc* instructions must be written, and considerable reprogramming may be required when a procedural embedding system is extended to cope with a somewhat new problem domain¹¹.

The Stanford Research Institute problem solver is used to control a mobile robot in tasks such as finding a box and pushing it through a doorway into another room¹¹. The core of the robot control system is an automatic theorem-prover that determines plans of action by proving theorems in predicate calculus. Knowledge of the world is expressed in statements in the predicate calculus, or, in other words, knowledge is assertationally embedded in the system. Assertational embedding has the advantage that the problem-solving system can be extended to new problem-solving domains without extensive reprogramming, since the principal changes can be made merely by changing predicate calculus statements. The theorem prover is a general purpose program that is independent of specific problem semantics, and this¹¹ provides us with a very clear example of generalist work. It is less clear how much generality there is in the interface between the real physical world and the assertational representation of it. In a generalist system the requirement for generality precludes the limitless sophistication that can be built into a specialist system *ad hoc* programming; and the problem of keeping abreast of changes in the world may be particularly acute in a generalist system¹².

Acquisition of knowledge

Whether knowledge is represented procedurally or assertorically, it must first somehow be acquired. Feldman and Yakimovsky¹³ have devised a system that automatically labels area of a photograph as "sky", "mountain", "grass", and so on. Indeed the system partitions a photograph into such areas. This system uses previous knowledge that in the world "sky" is above "mountain". Here, "above" is a relationship, and in partitioning a photograph the system exploits knowledge of many such relationships: Feldman and Yakimovsky have developed a general technique for using such relationships, provided that these are given to the system. The system has statistical learning capabilities but has no means of automatically acquiring knowledge of these relationships, for instance by processing unpartitioned specimen photographs of many different scenes.

This acquisition problem does not seem to be intractable, and indeed I have already investigated a related problem¹⁴, the first of a series in AI¹⁵. Although this work was admittedly abstract, primitive, and impractical, it did show that one can make headway with the segmentation of a pattern into parts, given as data only a set of unsegmented specimen patterns composed of different combinations of parts. A solution to this problem would seem, to my intuition, to be nearer to intelligence than would a segmentation technique that relied on a human to supply knowledge of relationships between parts. This also serves to illustrate my previous remarks about previous-experience input data.

For practical purposes of segmenting a photograph into semantically homogeneous areas (for example in aerial photointerpretation) there may be no advantage in automatic acquisition of relational knowledge that is used for achieving segmentation. Instead, this problem of acquisition may be left to those of us who do not forget that there is only limited information capacity in the genetic code by which the brain structure of an offspring is determined by a parent, and to those of us who would prefer to model the lower levels of practical intelligence before proceeding to the understanding of natural language by the machine^{9,16}.

Intelligence

In a top-down parsing algorithm, or any other kind of algorithm for that matter, there is no single instruction that is the "heart" of the algorithm. A top-down parsing algorithm is recognisable as such by its overall structure, in spite of the fact that it contains no tangible heart. A written word is recognisable as a combination of letters, despite the fact that it contains no letter that other words

do not contain. An algorithm that should properly be called intelligent may be recognisable by its overall structure, and "we should not let our inability to discern a locus of intelligence lead us to conclude that programmed computers therefore cannot think"¹⁷, but this does not imply that all problem solving algorithms should properly be called intelligent. The presence or absence of a heart, locus, or "blinding white light" is irrelevant to the determination of intelligence of an algorithm.

To Minsky "... 'intelligence' seems to denote little more than the complex of performances which we happen to respect but do not understand"¹⁷. To me "intelligence" denotes a very distinctive class of problem-solving processes, and I do not think that the word "intelligence" should be applied to radically different classes of problem-solving processes, even when these successfully solve the same problems.

Finally I shall hazard some guesses about the distinctive nature of intelligence. It seems safe to guess that, in the brain, intelligence is realised by a highly parallel computation using distributed logic. This computation could presumably be simulated on an ordinary digital computer, but perhaps awkwardly, inelegantly, and even impractically. Animals and people do not always seem to need the same sort of instructions that computers need, and I guess that in some cases intelligence is realised in the brain by a sequential process that does not use stored instructions: the process is non-programmed, not self-programmed, in that there is no stored program. I guess that in the design of the brain there are solutions to two of the salient problems of computer science: the mitigation of programming effort, and the organisation of parallel computation.

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Distributed computer networks

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How desirable and inevitable are large heterogeneous, distributed computer networks? In this article Professor Kirstein, whose group at University College London operates one node of the large international ARPA network, describes the motivation for setting up such networks and the problems which must be resolved before they are deployed more widely. Those problems are often more political and sociological than technical.

THERE is considerable argument as to what is meant by a computer network. Any large modern computer system contains several processors and for commercial or technical reasons these may be mounted in one set of cabinets. Nonetheless, they could still be regarded as a network, and here I shall define a computer network as a collection of computers, connected through communication lines to other computers or terminals. This definition will not please all the proponents of computer networks: some will consider it too narrow, because it does not include single collections of connected

processors; others will consider it too wide because it includes Star systems (Fig. 1). There is, however, a qualitative difference between the purpose, technology and potential of the systems which come under my definition of a computer network and single collections of connected processors.

Figure 1 shows the principle ingredients of a Star-organised computer network. A single computer centre (C) is connected through a data network (N) to a number of terminals (T). Each of the elements C, N, and T can have different levels of sophistication. The computer centre can be one processor or a set of computers at one site (perhaps interconnected, as shown in Fig. 1). The data network can be just the public switched telephone network (PSTN), the British Post Office experimental packet switched service (EPSS), a distribution system based on leased lines and privately owned switching computers, a set of leased multiplexed, telephone channels, or a mixture of elements like these. As an example, Fig. 1 shows a hierarchical leased network of central concentrators (CC), and remote concentrators (RC) connected to the PSTN. The terminals may be either simple keyboard terminals (or displays), or more complex computer work stations which control card readers, printers, magnetic disks and tapes as well as keyboard terminals. A key aspect of all these computer networks is the desire to increase the catchment area of the individual computer sites, and to make the computers available to people elsewhere.

In a very simple system, the C of Fig. 1 may be only a single computer, the N, the PSTN, and the T keyboard terminals; many T of the smaller time sharing bureaux are so configured. A single computer (H in Fig. 1) is not, however, sufficiently reliable; for better availability, multiple computers are incorporated in the computer centre. The PSTN is adequate for local access to keyboard terminals. To provide a larger catchment area at cheaper cost, however, the installation of concentrators (RC in Fig. 1) connected by leased lines to the computer site is more cost effective. The PSTN is restricted, in the UK, to 300 bits per second (b.p.s.), which is 30 characters per second in two directions simultaneously, or somewhat higher speeds asymmetrically (for example, 100–200 characters per second one way, 7 characters per second the other). Thus, to provide higher speeds (for example, for batch card reader–line printer terminals) leased line networks are requested. These lines can go direct to the central site, C, or to nearer concentrators (RC in Fig. 1).

Because the computer systems themselves were the least reliable components, it was unnecessary with the earlier networks based on single computers in central sites to put much sophistication in the data network. Such systems are still limited, however, to serving about 100 users simultaneously. To improve availability, multiple mainframes were incorporated in C. Moreover, performance was improved by introducing a certain amount of functional specialisation. For example, in both the London¹ and Manchester University regional computing centres, one machine is being used for the main large batch processing, and other front-end computers (FE) deal with the interactive traffic and submit work to the batch processor.

No commercial limit has been reached yet on the number of terminals that can be serviced simultaneously by a system with the topology of Fig. 1. Indeed, some of the larger time sharing bureaux have used it to service several thousand terminals simultaneously. Of course, in those circumstances the organisation of C is complex, with duplicate access to the communication lines, processors and files. These precautions guarantee the continued availability of resources in the event of failure of most individual subsystems.

The data networks are configured very carefully, with due consideration given to the connections of RCs to CCs, duplicate CCs, the optimum siting of RCs, and so on. Such systems must provide highly reliable access at least for interactive activities. The General Electric Network² and TYMNET³ are the best developed of this class.

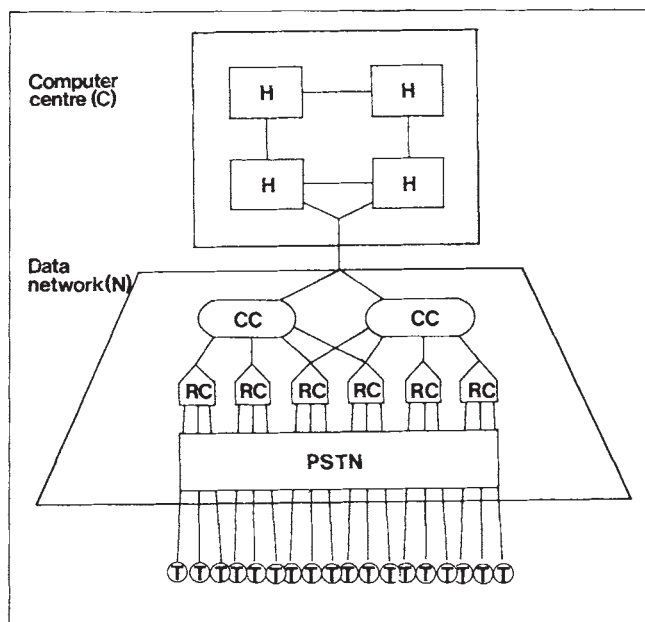


Fig. 1 Star-organised computer network. H, Hosts; CC, central concentrators; RC, remote concentrators; PSTN, public switched telephone network; T, terminals.

The availability of different processors in C (Fig. 1) allows a considerable degree of functional specialisation. One or more systems may be used only for interactive editing, others for access to specialised data banks, and yet others for very large parallel processes. One noteworthy example of this functional specialisation is the ILLIAC IV complex at NASA-AMES⁴. At last count it contained more than 30 computers, from those driving the 10^{12} bit store, controlling the communication functions, to those providing interactive file manipulation and controlling the 64 parallel arrays or processors.

There is one facility not provided in the systems of Fig. 1: geographical separation of the host (that is, the main service machine). For many reasons this distribution can be very important, geographically distributed sites may have a large local catchment area, so that only a small proportion of computing need, or should, be exported. A balance between gains from larger computing centres against added communication costs can make the topology of Fig. 2 most attractive. In practice, computing centres are often brought into being for local reasons, but cannot supply all local needs. In such conditions the topology of Fig. 2 is a natural outgrowth. Here, the individual host sites, C, may have the form of Fig. 1 in its simple or more complex embodiments, each site responsible for a distribution network, N, to its own customers, T. In addition, there is now a high level data network (HLDN) connecting the host sites. The terminals can be switched through the HLDN to other sites. Three networks which use this technique are the early versions of ARPANET⁵, the French Cyclades⁶ and the UK South-west Universities Network (ICNS)⁷. As a final level of sophistication, the terminals (T, in Fig. 2) can also be introduced directly to the HLDN, as on the present versions of ARPANET⁵ and TYMNET³. Obviously, practical networks do not always allow a neat classification into those of Fig. 1 or Fig. 2. For example, the General Electric network² is essentially of the type of Fig. 1, with a high level data network connecting individual computers in the computer site. The computers themselves are distributed in two centres, and could be spread over more than one network with no change of technology.

Using the networks shown in Fig. 2 it is possible to have a more rational growth policy. One centre can be expanded and some of its surplus used to delay the need of expansion at other sites. Centres not willing to forego their own computer systems may be much more willing to supply a highly specia-

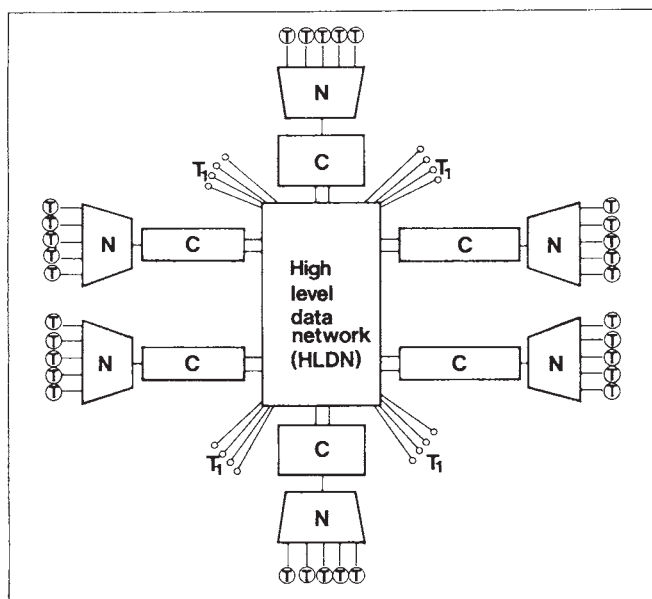


Fig. 2 Distributed computer network. HLDN, High level data network; other symbols as in Fig. 1.

lised proportion of the computing requirements for a whole community. The difficulties posed are not technical but political and sociological.

The national communication carriers would like to offer the maximum portion of data network traffic; at the least, they will always lease transmission channels. Many are proposing special data networks which also provide any switching and multiplexing required. Examples of planned offerings in this area are the US TELENET⁸, the UK EPSS⁹, the French TRANSPAC¹⁰, the German EDS¹¹, and the Nordic network¹². The communications carriers would like to separate out the data network portion as much as possible from the rest of the network, and some of the commercial interests (for example, TELENET and TYMSHARE) would like to provide them as commercial services.

With the systems of Fig. 2, very careful definition is required for the communication protocols at different levels. When the different computer systems are from the same manufacturers, as, for example, in the South-west Universities' network, the problems are easier. But when the systems are different, as in ARPANET⁵ and Cyclades⁶, very careful design and implementation is required¹³. Sometimes, as in the UK EPSS, the carrier defines one portion of these protocols, but in such

cases it may still be necessary for those responsible for connecting in computer sites or terminals to define other levels¹⁴.

The data communication network may be very heterogeneous itself. Besides containing a very hierarchical structure, it will in the future include new technologies. In the US ARPA has been used to investigate the use of satellites for broadcasts¹⁵.

A set of experiments to investigate this technique, to be carried out jointly by ARPA, the British Post Office and a number of research organisations (including my group at University College, London (UCL)) started in the summer of 1975. ARPA has also been used to investigate access to the local nodes by packet radio¹⁶.

The ARPA computer network and UK usage

In the ARPA network, the sites are connected together by a communication subnetwork (ARPANET), 50×10^3 b.p.s. (Fig. 3). This comprises leased lines (usually at 50×10^3 b.p.s.) connecting communications computers called Interface Message Processors (IMP)¹⁷. The system has extra connections to permit alternate routing in case one node becomes inoperative. The host computers (H) are connected to the IMPs, and may be local or remote; in the latter case the connection between the IMP and the host is by a leased telephone line. In some cases only host computers are attached to the IMP; in others, terminals can also be attached. The latter set of communication computers are called terminal interface message processors (TIPs)¹⁸.

The important attribute of this network is that messages between hosts are split up into packets. Each packet is up to 1,000 bits long and contains the full information about its source and destination in its header. The IMPs contain the information on how to route from the source to the destination. Cost saving arises from the possibility of using a much smaller number of communication lines than would be required in a system in which each pair of nodes was joined by a pair of lines. The fact that the destination information is contained in the header saves the time and overheads required in setting up a virtual circuit. It is possible to use high-bandwidth lines with appropriate cost savings, and achieve a maximum transmit time between sites, in the absence of satellite hops, of 200 ms for single packets of 1,000 bits. ARPANET now stretches to Hawaii, Norway and London (Figs 3 and 4).

The connection of hosts sited near the IMPs to ARPANET relies on a special hardware interface because of the lack of standard communication interfaces. This policy has also been used in later developments of ARPANET and similar subnetworks^{5,8}. A considerable body of software is required in the hosts if this heterogeneous collection of computers is to be used in a somewhat homogeneous manner. There is a network control program, which embodies the procedures agreed for a process in one host to connect to a process in another. There

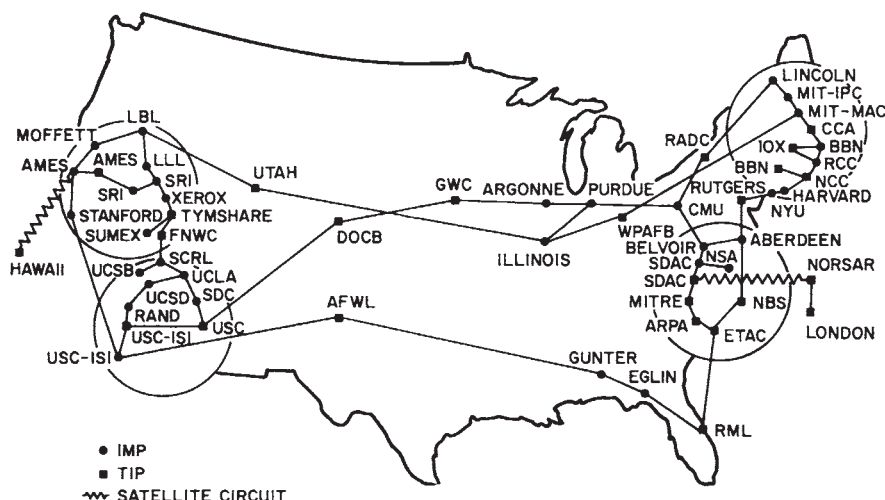


Fig. 3 Geographical map of the ARPA network (April 1975).

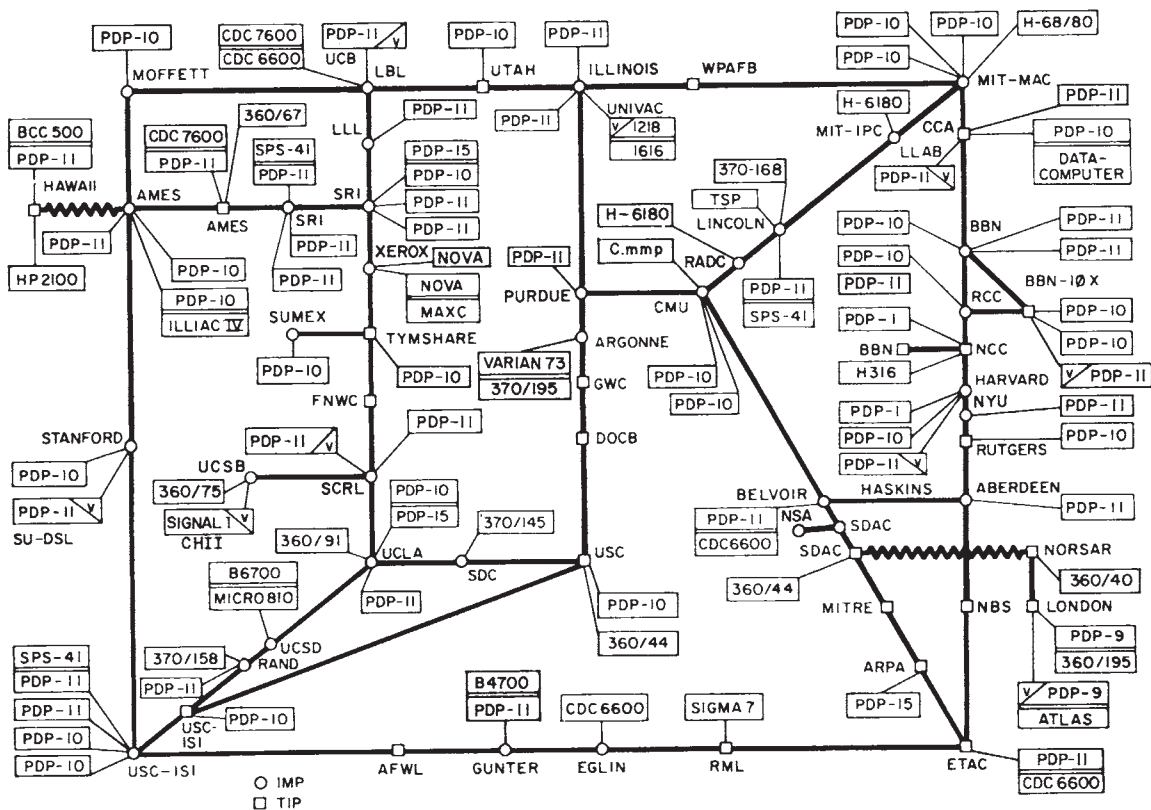


Fig. 4 Logical map of the ARPA network (April 1975).

are protocols for network virtual terminals and file transfer. Even higher functions such as Graphics and Mail Transfer have been standardised¹³. In this context the word 'protocol' means an agreement by the participating sites to implement programs which will accept the standard format of data and control for the relevant functions.

The ARPA network has not gone as far as possible in standardisation. Though there have been discussions of network-wide job control or command languages, and even editors, these have not been implemented in practice. This was partly because of the lack of effort available, and partly because of the increasing difficulty in agreeing on standards for the higher functions. In our experience about 8,000 words of code (in our system 4,000 words resident) are required to implement these protocols.

To operate such a network it is essential to have a network control centre¹⁹. Extensive information on how to use the systems must be made available. Much of this is kept on-line on the host computers; however, a special facility, the Network Information Centre, has been set up for this purpose. Lack of funding has not allowed this to be kept up properly leading to some problems. In the UK, my group is providing a documentation and advisory service to other UK groups using the network. For this purpose we have arranged to make many documents available in a special collection in the British Library (Lending Division).

It is very difficult to organise efficient advisory and co-ordination activities in such a network without good, simple message services. For the research most of the hosts have instituted some facilities for exchanging text messages between the users. The procedures have been standardised, so that the messages can be sent to other sites. Simple composition and retrieval facilities have also been provided (for example, simple editing, ability to append lists of addresses, single line titles, procedures for listing only the titles of messages, and so on). In addition, there are facilities for linking terminals together to enable two or more terminals to communicate in

real time with each other—even though they are attached to different hosts. This is very interesting and complex²⁰, though its uncontrolled use would be against the regulations of the PTTs. In the context of the international ARPA network its use is only permitted for specific purposes concerned with the computations in progress. An important lesson to be learned from the ARPA network is that the communication sub-network, ARPANET, which is the entity which will in future be provided more by the PTTs (refs 8–12), is only one part of the infrastructure required to make such networks function effectively. The subnet, because it is usually implemented by one organisation, should proceed smoothly. The development of the rest of the infrastructure can be much more time consuming.

In the context of scientific multi-user networks the types of use which are being made of the hosts will be of interest. The ARPA network is being used operationally for a large number of projects. It is the principal means of entering jobs into the Illiac IV computer; a substantial proportion of the use of MULTICS, the UCLA IBM 360/91, and many of the TENEX sites work through this network. Among the substantial applications of ARPANET are the acquisition and analysis of seismic data, large scale climatic modelling, the administration of ARPA projects, and the coordination and cooperation of hundreds of research programs. A partial list of projects which used the UCL link of the ARPA network during 1974 is given in Table 1. This list encompasses a broad range of subject areas, research institutions, and government departments; the uses are discussed much more fully elsewhere²¹. Most, however, involve cooperative activities between different research groups in the UK and USA. Sometimes the cooperation involves shared use and development of the same programs (for example, University College and the Royal College of Arts, Table 1); sometimes the computer resources of one group are traded for those of another; sometimes one group has access to a computer system belonging to another so they can develop software of mutual interest

(for example, Utah's use of the RL 360/195). Sometimes the incentive to cooperation is access to data bases built up jointly or just accessed by more than one group (for example, the Blacknest Seismic Data Base and the British Library use of Medline)²², sometimes the main motivation is just to have the groups share their research experiences more promptly and completely (St Bartholomew's Hospital and Southampton University's use of the teleconferencing system Forum)²³. Of great importance here is the unpredictability of the uses. In 1971, when the UCL project was proposed officially, there were eight proposed users (all in computer science); of these only half ended up using the link in practice. All the others in Table 1 starting applying (and are still applying at the rate of about two a month) once the link was established.

Motivation for computer networks and their development

There are six fundamental reasons for preferring the use of a network to that of a local machine.

First, the cost of running a particular job may be substantially lower on a larger machine than on a smaller machine, even allowing for communication costs. For that reason, a larger computer than is required for local computing, with a suitable data network to enlarge the catchment area, may be economically attractive. That is the rationale behind the establishment of the University Regional Computer Centres by the UK Computer Board. Wells²⁴ has commented that the cost of processing goes down as the square of the computer power, whereas the cost of file storage varies linearly for any particular machine type.

Second, with a large collection of computers in a network and corresponding catchment area, it is possible to make smoother increases of capacity to meet demand, and to provide better reliability and availability. This is one of the rationales behind the TYMNET and General Electric networks.

Third, with a large catchment area, covering many time zones, the diurnal demand cycle means that the peak computing requirements of some of the users will come at the low activity periods of others. The effect is very pronounced in

the networks which span North America, Europe, and the Far East. Even with Europe and North America covered alone, the effective peak day is 18 h long.

Fourth, specialised resources like data bases, programs, or special hardware, which would otherwise not be available, can be accessed. This is the major motivation of many of the users of service bureaux and stock exchange dealings also come into this category. In the scientific field seismic and weather data bases, bibliographic data bases, large data bases developed for high energy physics experiments and molecular structure come into this category.

Fifth, for research or other groups to cooperate on the same or related projects they may need access to each other's data or programs. They may also need to share messages with short response times, or even in real time, with several other people at different sites.

Finally, there may be a requirement of computing capacity at each site; the network environment enables one site to increase capacity beyond the local requirement and export some capacity to others in the network. This leapfrogging technique enables all the sites to vary their systems progressively with minimum dislocation and short term over-capacity. It enables different centres to specialise on specific areas, while keeping all areas available to local users. The South-West Universities Network works like this.

For some applications the type of network used is immaterial, but traffic considerations and the cost of communications make those like the ARPA network the most economic. This is particularly the case when there is a genuine need for local computing—often resulting partly from the difficulty in attracting good staff without a local computer.

A closer look at the costs of the different applications of computers, shows that there are often considerable advantages in dedicating computer systems for specific applications or types of service. One reason for this is the pure hardware and software cost of providing the computation for each item. Another is to avoid the cost of duplication at development and storage stages of the packages, and to provide the relevant advisory facilities.

Table 1 Partial list of UK project using the ARPA computer network through the UCL node (March–December 1974)

UK organisation	US partner	Purpose
Blacknest Research Establishment	Lincoln Laboratory, MIT; NOR SAR, Norway	Cooperative construction and analysis of seismic data bases
British Library (Research Division) with 15 UK centres under their sponsorship for a specific experiment	National Library of Medicine	Experimental comparison of MEDLINE, a medical bibliographic information retrieval system, with a similar UK system
Cambridge University	Utah University	Development and comparison of algebraic manipulation system
Culham Laboratory	NASA-AMES	Application of ILLIAC IV to magneto-hydrodynamic computations using symbolic manipulation techniques
Royal College of Art	Harvard, MIT	Development and evaluation of space-planning systems in architectural design
Rutherford Laboratory (including its user groups at Oxford and Surrey Universities)	Illinois University Maryland University Harvard University Chicago University	Exchange of software and data for theoretical high energy physics calculations
St Bartholomew's Hospital Medical School	Brookhaven National Laboratories	Teleconference on human response to sulphur pollution
Southampton University Politics Department	University of California, Santa Barbara	Teleconference on political simulation models
University College London, Department of Statistics and Computer Science	Stanford University Bolt, Beranek and Newman	Experiment on the requirements for interconnection of computer networks

A rational policy of computer provision for, for example, the university and research communities would therefore include some local, general purpose, computing facilities (probably not connected to a national network), some regional, large scale, general purpose computing facilities (part of a regional network), and some specialised facilities (clearly, part of a national, and in some cases an international, network). Some types of computation may be done best in commercial bureaux, and even that should be permissible. Together with the build up of computer facilities in this way, some control is essential to ensure that the computing is done on an appropriate facility—and that the operators of facilities do not duplicate unduly activities already carried out well elsewhere. Such a computer policy raises many problems: on the one hand it is ludicrous to develop a complete expensive body of programs and data bases in a specific area as a general service, if another adequate set exists already accessible by the same user community; on the other, one should not prevent progress in providing new and better facilities. Sometimes, such improvements can be made in an evolutionary fashion but at others a completely new system is required.

Using any network on a large scale requires resolution of 'balance of payment' issues. It seems a fact of life at the moment that institutions want the maximum facilities locally, and resist resources going elsewhere. They prefer to be net exporters—and to be paid to export. For the concept of specialised resources to work, means must be found for financing their development and utilisation—even at the cost of local generalised facilities. Resolution of this problem becomes progressively more difficult as the computers become wider apart.

In the UK, the Science Research Council has succeeded in this respect with the High Energy Physics Community. Regional University computing centres have been installed for large scale general computers. Only now is there discussion of a 'computer aided design' centre for the universities (though there is one in Cambridge run by the Department of Industry). This concept should be carried much further—but needs a widespread data network to permit its implementation. A candidate for this network should be in existence soon in the UK with the development of the Post Office's Experimental Packet Switched Service⁹. Technically, international usage is possible, and there are communities who desire and require such use; it must be resolved, however, whether the appropriate payments or regulation mechanisms can be devised.

There is a further serious question to be resolved on network usage, and that is a complex mixture of regulatory and economic issues. No tariffs for EPSS have been published. It

is clearly cheaper to set up private user data networks (like those of Figs 1 and 2) than to use only the facilities provided by the Post Office—though this is not necessarily so in the US where 'value added networks' are permitted²⁵. In the UK, and even more internationally, there are severe restrictions on which users may join together in a private network. There are, for example, several very large bibliographic data bases owned and operated by different organisations²². These fall clearly into the description of the 'specialised resources' mentioned already. In the UK (and in many other countries) a private data network operated by one organisation is not permitted to provide the public access to several such systems.

This problem is even more difficult if the common user service is to be provided internationally. We can expect a continued dialogue between the PTTs and the service suppliers and user communities to modify tariff and regulatory policies in the light of economic pressures and technical advances.

Technology now exists for large scale, distributed, heterogeneous computer networks. The most common method of implementing them is by a separate data network with host computers attached. There is still some discussion as to what should provide the data networks, what facilities they should contain, and how they should be tariffed. The main bar to a wider deployment of such networks is the need to resolve the organisational, regulatory and financial issues they raise.

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Computers and the printing industry

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As page composition from digitised information replaces the manual assembly of type, the printing industry struggles to absorb a technology in which the compositor becomes a computer operator and the production controller a systems analyst.

CRITICS of print technology are fond of pointing out that if Caxton returned to the industry today he could walk back into his old job with the help of a fortnight's refresher course at the London College of Printing. Certainly it is true that

Gutenberg, who developed moveable type in the fifteenth century, would have no great difficulty today in locating a composing room which used materials and crafts recognisable to him, whether in his native Strasbourg, in Britain or the USA.

For many years it seemed strange to technicians outside the print industry that something like an office typewriter could not be put on-line to a computer and thence to the printing process. In 1867 a Milwaukee editor, Christopher L. Sholes, patented the machine which gunmakers E. Remington and Sons manufactured as the Remington Model 1, yet a century later even the simplistic use of an IBM typewriter driven by punched tape or on-line to a small digital computer is an innovation in some print houses.

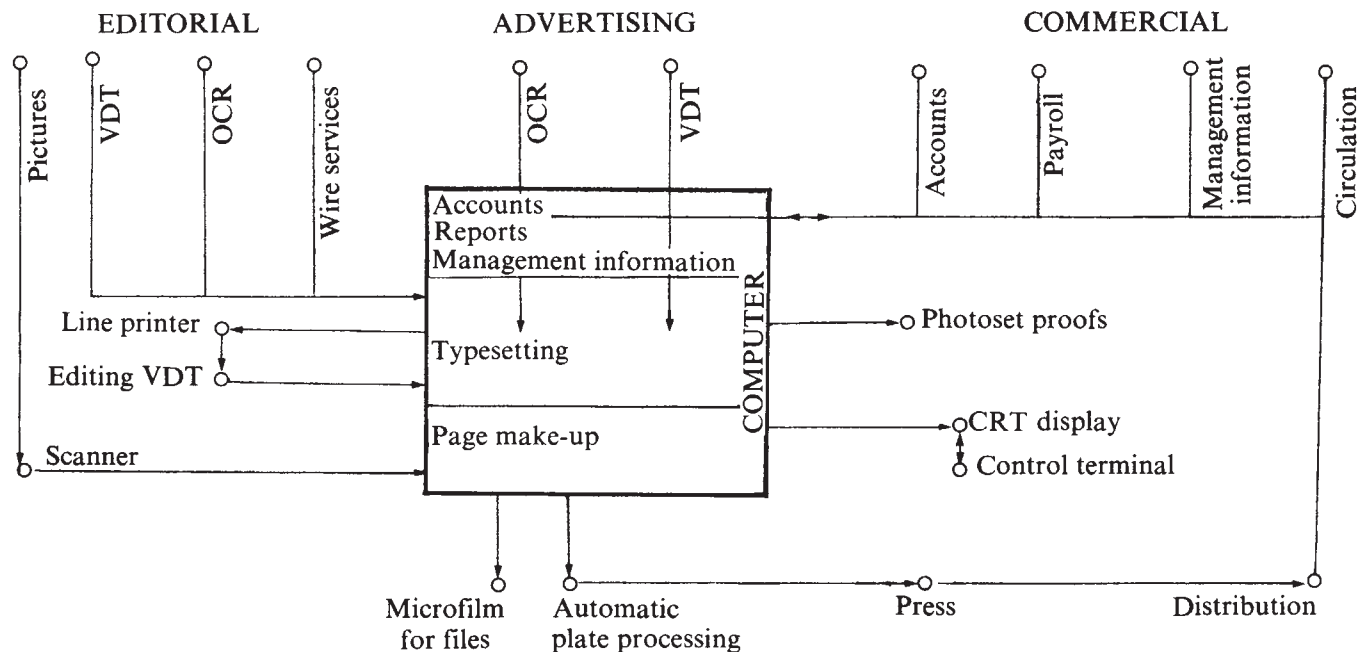


Fig. 1 A theoretical typesetting and page make-up system for a daily newspaper. Picture input is the present obstacle.

Countless frustrated authors and publishers have condemned the mediaeval technology of typesetting while at the same time feeling some affection for the traditional techniques which for centuries defied any degree of mechanisation. It is therefore in some ways sad but probably inevitable that the computer in print is ultimately destined not to refine the traditional craft skills but rather to replace them.

If printing is considered a two-part operation comprising first, the processing of words into a printable form and, second, the manufacture of a readable product (which is possibly in the long term an unnecessary breakdown), then the first part at least can be achieved at present by computer-driven hardware controlled by technicians who are not necessarily trained printers.

The feasibility of the jump—after 500 years of technological stagnation—from techniques which involve the individual collation by hand of lead letters or lines to systems which effectively dispense with manual skills altogether can only be appreciated in the context of the second part of the production process, the printing operation, since this largely governs the form of origination necessary.

Caxton and Gutenberg, on their reincarnation as twentieth century printers, would print by the letterpress process, involving the pressure of an inked relief image against the paper. In this case, the opportunity for the use of a computer to progress beyond mathematical problems of letter assembly (such as word and letter spacing) is qualified by the unavoidable complexity and size of the machine which would then be necessary to assemble automatically a page of relief metal. Consequently only limited mechanical assistance of any sort has proved to be economic in metal typesetting. It is possible and usual, of course, to cast type in 'hot metal' by machine, but normally this process ends with the collation of lines of type, after which pages have to be made up skilfully by hand.

The Monotype, which automatically casts individual letters in lead on instructions from a keyboard perforated paper roll was invented by Tolbert Lanston in 1887; Mergenthaler's Linotype, which casts lines of lead type in a semi-automatic process from directly keyboarded commands, went into production a year earlier and became the standard newspaper setting machine. Still in general use as updated models throughout the world, both machines retain the appearance of having been created by Heath Robinson after a heavy night out.

The Linotype and Monotype represent the most commonly used methods of mechanically casting metal type but manual expertise is needed both in operating complex keyboards and in making up pages from the set type; multiple installations are necessary if any volume of setting is to be achieved.

Computers are successfully used to produce perforated tape to drive linecasting machines, but the metal output is too restricted to allow much application beyond the production of lines of justified type.

Although letterpress has inhibited computer potential, the commercial development of offset lithography has encouraged it. The offset lithographic printing process was discovered accidentally in 1796 by Alois Senefelder who was working on a method for reproducing music notation, but it was not until well into the twentieth century that extensive commercial use was made of the process. Lithography uses a planographic plate which is developed photographically, so anything which can be photographed—even typewriting, for example—can be converted into a printing plate.

The lithographic technique involves the use of pre-sensitised plates exposed through a film (negative or positive) of the page of type and illustrations. Development of the exposed plate leaves the non-printing areas hydrophilic with the printing surfaces ink-attractive. Judicious application of water, then ink, to the cylindrically mounted plate allows the transfer of the exposed, inked area to a revolving rubber blanket and thence to the paper.

Replacing metal

As offset lithography slowly replaced some letterpress installations, the inconvenience of metal type became increasingly burdensome. Since input to the lithographic plate-making process was in the form of 'flat artwork' ready to photograph, metal type needed proofing on high quality paper before it was ready for the camera.

Alternatively, other methods of composing type could now be used, and as paper or film—as opposed to metal—output was required from the composition process, electronically controlled typesetting machines became convenient and economic.

Two main systems have been used extensively: a 'near print' process which uses a typewriter-based device such as the Friden Justowriter or IBM Selectric to 'type' setting as a straight

manual operation or from coded tape, and a photographic process in which the typesetting is produced as film or a bromide print. Both methods can use binary logic to solve the arithmetical problems involved in setting letters of varying widths, but for various reasons, including speed, versatility and the suitability of the end product, photo- and filmsetting have made the widest use of the computer. It is therefore relevant to look briefly at the mechanics of photosetting.

The early filmsetters (for example the first Monophoto) were mechanically based on hot metal casters with matrix cases of typographical characters on film replacing the caster's case of 'moulds'. A quartz-iodine light source projected a selected character on command on to photographic film. Most electronic photosetters, on the other hand, store characters on disks, drums or grids. Disk or drum stores rotate continuously and characters are illuminated by xenon flash tubes. Grid characters are exposed in a stationary position. The sizes of characters may be controlled optically by moving or changing lenses within the exposure routine or by storing character negatives in varying sizes.

Such photosetters may be quite sophisticated in themselves, with special-purpose minicomputers controlling the word and letter spacing, or they may be 'slave' units operating on instructions from a separate computer. Input is usually by Qwerty keyboard, either direct entry or off-line, using punched tape. If a small photosetter is using its own minicomputer, keyboards will normally be off-line, since its output speed will permit the consecutive acceptance of tapes from several keyboards while its computer will not have the power to store or process several inputs concurrently.

The fastest photosetters, mainly evolved in the past three years, use a cathode ray tube (CRT) to recall digitised characters which can be kept on file in a backing store. CRT photosetters are very fast and under intensive development, and are rapidly becoming economically viable for medium-sized printers.

Justification routine

The extent to which computer power is used in typesetting depends on economics, speed, volume of work, complexity of material processed and the number of ancillary operations involved.

Justification was the first and most obvious task to set a computer. By tradition, most printed matter, unlike typewriting, has proportionately spaced characters (that is, letters of varying width) and a straight (or 'justified') right-hand margin. The justification is achieved by varying the spaces between words within set minima and maxima. These parameters are stored in the computer memory together with data on character widths and line lengths ('measure'). Justification can then be achieved rapidly by subtracting from the measure the width of each

Table 2 Capital costs of a hot metal composing system, a conventional photosetting system and a practical ideal system

Hot metal £	Photosetting £	Practical ideal £
292,269	240,400	448,120

character as it is read, running back to the last space when the line overflows, and distributing through the inter-word spaces the total value of the space removed from the line end.

If the criteria for inter-word spacing do not allow this 'infilling' in a given case, then hyphenation is necessary. Computer hyphenation has been the *bête noire* of many photocomposing systems. The problem is that although it is possible to hyphenate by logic, there are, as in most grammatical rules, exceptions, and they are really too numerous to ignore. There was a short-lived move by some newspaper proprietors using photosetting to impose irregular hyphenation on their readers, but they discovered that the irritant factor was too great. Some systems enable the operator to decide hyphenation by displaying the word, but obviously delays of this nature are not ideal and the present practice is for computers to store an 'exception' dictionary, the routine being to search the dictionary for the word and use the recorded hyphenation break if it is there, or otherwise to hyphenate by logic.

With short-measure work such as newspaper setting, 50% of the total setting time¹ can be spent justifying by traditional methods, so keyboarding in an unjustified mode and leaving spacing and hyphenation activity to the computer has obvious attractions in terms of speed. The resulting possibility of boredom for the keyboard operator can, however, be a problem.

Once the attractions of computer justification were established, larger machines began to tackle fairly obvious tasks which needed more memory, considerable random access or more sophisticated programs: 'formatting' by the use of limited command key strokes to produce commonly used typographical combinations; tabular composition; scientific setting using a wide variety of characters; mathematical composition with superior and inferior characters, special symbols and variations from the base line of the type; the acceptance and re-formatting of wire service material, and so on.

Optical character recognition

Methods of input were scrutinised as the new technology threw into focus the inefficiency of 'double keyboarding'—once by the author or his typist and then by the typesetter. Optical character recognition (OCR) is one solution with which the newspaper industry in the USA, Britain and Europe is being wooed. Like most flirtations, this is running a bumpy course, but the principle is sound enough.

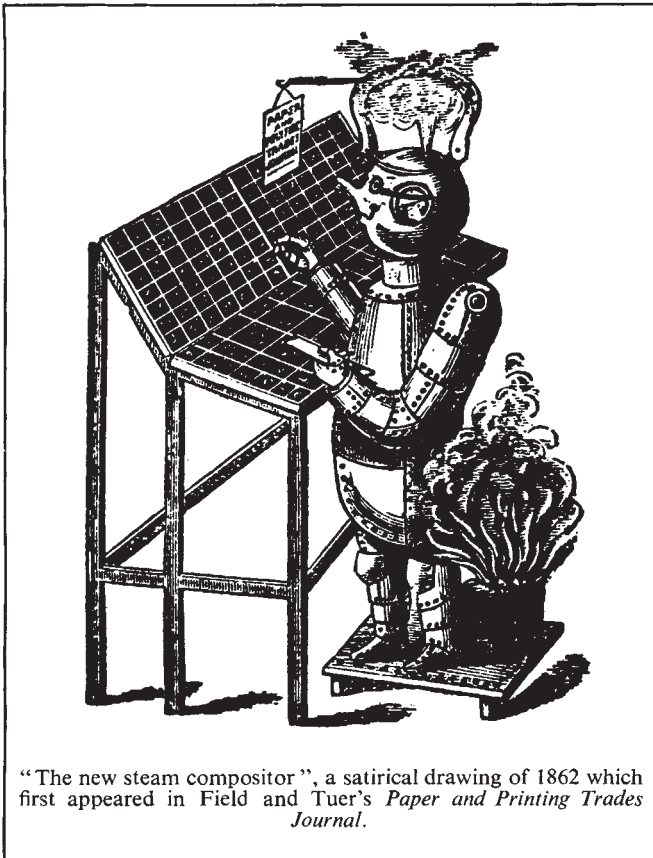
Typewritten manuscript is scanned by a 'reader' using photocells and the character which is recorded digitally is compared with master images held in binary form in the memory. Imperfectly formed characters will not be recognised on comparison with the memory data so fairly strict control of typescript quality is necessary. Specified typefaces must be used and carbon ribbons are used so that an even impression is made. Character recognition may still be thwarted by dirty typing heads, marks on the paper and so on, in which case the machine displays the offending line on a CRT with a query device such as a tick over the unrecognisable character.

Post-recognition logic may be very sophisticated with the acceptance of extraneous characters or character-combination codes for instructions such as 'delete previous word' or 'delete previous line'—given detailed code command programs and wide line spacing to allow for insertions, extensively edited copy can be accepted. Notes can be made on manuscripts with 'non-reading' pens, so most combinations of writing—editing—revising are possible. Output is normally to punched tape which can then be forwarded to the filmsetting computer.

Table 1 Running costs of a hot metal composing system, a conventional photosetting system and the practical ideal system

a Present running costs per year			
	Hot metal £	Photosetting £	Practical ideal £
Depreciation	15,048	29,580	68,040
Labour	209,962	183,130	85,179
Other costs	21,400	19,285	18,060
	246,410	231,995	171,279
b Forecast changes* in running cost indices due to rising labour costs			
	Hot metal	Photosetting	Practical ideal
Present	144	135	100
Two years	156	146	105
Five years	178	165	114

*Taking present 'Practical ideal' as 100 and assuming labour costs will rise 5% more per year than Depreciation and Other costs shown above, the changes in relative indices at two years and at five years due simply to this extra 5% in labour costs would be as shown.



Typewriter keyboards do not comprise a wide range of characters so the type of text which can be processed is restricted. Classified advertisement typesetting has been handled efficiently by OCR with the 'tele ad' girl who takes the advertisements by telephone producing the typescript which will become the typeset advertisement.

Extension of the use of OCR to general purpose setting is controversial, especially in newspaper work. If reporters can operate typewriters with the accuracy necessary for an OCR reader they can probably operate keyboards producing punched tape for the computer or sophisticated visual display terminals (VDTs) on-line to the computer.

Cost is an inhibiting factor, and so is the trade union viewpoint, but it is likely that for editorial work VDTs will be increasingly used in the editing process. Copy which has been sent to the computer whether by OCR, punched tape or other means, can be recalled to a CRT unit and edited—re-written if necessary—by keyboard and scroll devices, then restored. The implications for up-dating and 'editionising' on newspapers are significant.

An interesting sidelight on the output side is the increasing interest in computer output microfiche (COM). If typesetting has been digitised within a computer (or on magnetic tape for that matter) it can be projected on to a CRT and produced as microfilm or microfiche; in some industrial and academic applications the productions of 'hard copy' (paper output) can be eliminated and microfilm produced as the only form of origination. (The use of microfilm as computer input (CIM) is possible with a special OCR unit.)

Better hardware

The original typesetting minicomputers were small, simple units with no peripherals for storage. The tendency is still to use minicomputers but as additional applications have been spotted so the need for more random access has prompted the use of more and better hardware. Disk storage and magnetic tape are now widely used and the range of input and output devices has multiplied impressively.

Some of the new applications are closely linked with the typesetting function. When setting an index, for example, it saves considerable time to set from copy presented in random sequence and leave the alphabetical sorting to the computer. The index for *Nature* is handled in this way.

Other applications are less obvious. One of the most useful developments for newspaper houses has been the joint classified advertisement setting and accounting programmes. In a typical arrangement, classified advertisement information is input by OCR to create a master classified file which in the case of a daily newspaper is output daily for publication. The necessary data are included in each input to generate an invoicing and reporting cycle.

The master file is updated daily to carry forward advertisements which are booked for more than one insertion and delete those which have expired. The computer will sort into classifications and in some cases sort alphabetically within classifications. About 32 k of memory and reasonably extensive disk storage is needed to include this sort of operation.

In July this year a team from Portsmouth and Sunderland Newspapers in the UK completed a survey² into the 'practical ideal' for a provincial daily newspaper composing systems, based on equipment in use in the USA, the UK and Europe. Composing room staff would be "much reduced", said the report. For the size of newspaper considered, editorial copy would need 12 VDTs for input and a further 7 for sub-editing purposes. Copy which was written by reporters without direct access to the VDTs would be typed on machines with OCR heads and input by way of OCR readers. (See Fig. 1 for a schematic layout of a similar system.)

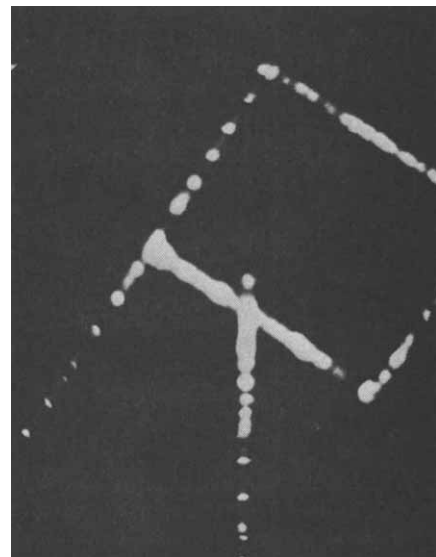
The advertisement department would need 11 VDTs (including one for the supervisor); non-VDT copy would be input by OCR. Some copy would need inputting without instructions, being recalled to the VDT for typographical display. Editorial and advertisement systems would be independent but each could back up the other.

Costs and the future

The costing aspect of a hot metal system, conventional photo-setting and the practical ideal is shown in Tables 1 and 2 (from ref. 2). When extended to show the cost of increased pagination, the practical ideal is highly advantageous.

In big newspaper houses and in some large commercial printers the practice is now to centralise typesetting, accounting,

Fig. 2 A magnified 'R' reproduced by lithography from a laser-etched litho plate developed by the London College of Printing research group.



payroll, circulation data and office uses in one large computer, but the outlay is prohibitive for the smaller company.

Complete page make-up within the computer and processing through to the platemaking stage without manual intervention is the ultimate aim of much print research. With simple formats, such as those in books, this is already in general use but with complicated newspaper pages there are problems.

Make-up of a page is possible on a large CRT but the input of graphics to a totally integrated page make-up system is still in its early stages of research. Theoretically the techniques are already available. Image data processing has entered the commercial market in various guises.

The Dicom Corporation of Minneapolis produces a series of 'digital image recorders' and 'image digitisers' which can store photographs in digital form and then print them back on to film. The picture is considered as a pattern of thousands of small patches of uniform brightness—'picture elements' or 'pixels'. It is scanned to sample the light intensity of each pixel, recognising a maximum of 256 intensity levels. This information is then digitised. To reprint the picture the information is recalled by focusing a CRT spot on to film, position by position, exposure time varying with the brightness needed.

The extension of this principle to the graphics area of full-page composition is obviously possible but a very high speed of input will be necessary and a vast amount of random storage will be needed for an operation of any size.

The general view seems to be that highly sophisticated full-page make-up is feasible but likely to be extremely expensive. At the London College of Printing (LCP), William J. Jones, the head of Pre-Printing Processes, and Dick Bowmaker, who runs the computer composition section, believe cheap hardware is the key to the next move in this area. The software, they say, is possible and the techniques available. Given the ever-accelerating development of inexpensive systems engineering, we are due for the next step. Expense is, as ever, the obstacle.

The LCP is intensely aware of the critical stage print technology has reached. Dr D. J. Morantz, the Deputy Principal, says the college is "endeavouring to secure facilities to research and teach the new areas of print and communications technology". Morantz himself leads a small research team at the college and has recently developed a technique for using a laser to etch litho plates.

Lasers have been used successfully to engrave plates for the gravure process, which prints from recessed images. For litho a subtler approach is necessary in view of the need to create a surface etching suitable for the rather difficult chemical requirements of the process.

The LCP team has used an argon ion laser on a specially coated 'plate' from which they have subsequently printed by lithography with promising results (see Fig. 2, for example).

They are now looking at the production of a direct coloured image by the same process and have succeeded in etching coloured images using specially formulated coatings. To project the idea, the production of a laser-etched plate direct from digitised data might be possible.

The new print technology is quite clearly a major headache for the print unions. In the USA there have been significant local breaks with print unions which could not come to terms with new techniques. Peter Watson, Digital Equipment Corporation's North European Technical Representative, estimates a move in the USA from 70% of newspapers unionised in the pre-computer days to 40% today, with the trend continuing.

In the UK, there have been minor breaks with the principal unions concerned and some serious disruption of production as new systems have come in. The National Graphical Association (NGA), the main union concerned, is inclined to take a fairly enlightened view at national level. "We've got to come to terms with the computer", says Roy Pearson, NGA National Organiser. "I think a careful programme of re-training is one of the answers. We accept that some compositors must become computer operators and even programmers."

But as Pearson admits, the problems in practice, at local level, are formidable. Computerisation of the national press composing rooms (which is now an economic necessity) must imply a massive fall-out of compositors, and potentially boring jobs for some of those who remain. None of the English national newspapers has yet reached an agreement on the full use of computer controlled composition. The human problems are certainly likely to be bigger than the technological ones.

This necessarily short review has deliberately ignored large areas of the printing scene. It has not mentioned colour processing or machine control, in which computers are used effectively. It has omitted details of developments such as the use of photopolymer plates which make photosetting methods suitable for the letterpress printing process.

The main purpose has been to sketch out the areas in which the skill of the 'printer' is being wholly or partly replaced by the computer, for this is the most significant aspect of the subject from a technological and human viewpoint.

I have therefore concentrated almost exclusively on the role of computers in composition (although it is likely anyway that the composition process will eventually extend through to the platemaking operation). This is the area in which the most dramatic modifications are taking place, the part of the industry which is changing from craft to computer

¹ Holland, F. C., *The Role of Computers in Photocomposing* (PIRA Information and Training, Leatherhead, 1974).

² Miles, W. E., Wrightson, W. S., Penketh, D., and Lister, K., *Practical Ideal System of Copy Origination in Medium Sized British Provincial Newspaper* (Portsmouth and Sunderland Newspapers Ltd., Portsmouth, 1975).

Minicomputers are bursting out all over

Roger Woodham

The pre-eminent position of the central computer installation to which programs and data are submitted, to emerge hours or days later, is being challenged by the minicomputer, which offers considerable computer power to individuals at the time and place they require it.

ASKING for a handy definition of a minicomputer is rather like asking the length of a piece of string: few people are prepared to commit themselves except in the vaguest of terms.

Yet minicomputers have completely changed the face of computing by making available to individuals—be they scientists, medical personnel, civil engineers or teachers—the kind of personal, 'hands-on' computing facilities that the large mainframe computer, in its cloistered and pampered surroundings, can never provide.

When pressed, however, people in the computer field will generally classify a particular computer as a 'mini' on the basis of its price. Broadly speaking, a minicomputer system costs between £1,500 and £50,000, at which price it turns imperceptibly into a 'midi', costing anywhere between £50,000

Table 1 Principal minicomputer manufacturers

Company	Market share 1974 (%)	Minicomputer revenues (\$ million)
Digital Equipment	35	410
Hewlett-Packard*	13	150
IBM*	12	145
Data General	8	92
General Automation	6	65
Honeywell	4	50
Varian Associates	3	34
Interdata	3	30
Modular Computer	2	26
Computer Automation	2	22
Systems Engineering Labs	2	18
Microdata	1	15
Digital Computer	1	10

*Includes internal installations.

and £150,000; the midis, in turn, can be divided off from the 'maxis' or 'majors', which cost more than £150,000. At the other end of the scale there is the microcomputer, the 'computer on a chip', which may cost as little as a few hundred pounds but which is so much more than a glorified hand calculator.

Growth industry

Most people would associate the name DEC (Digital Equipment Corporation) with minicomputers, and certainly that company makes the lion's share of all minicomputer sales (see Table 1). But then it was a trend setter as long ago as 1958 and has been selling its PDP series, among others, ever since. Data General, on the other hand, is the prime example of a blossoming newcomer, founded only in 1968 by a former employee of DEC who put his own ideas on the development of minicomputers into practice on his own account when DEC failed to adopt them. Since then Data General has gone from strength to strength, doubling its annual sales (which have totalled 20,000 machines in its seven years of life) every year.

During the past eight years or so, the costs of minicomputers have dropped considerably, as the price of electronic components to carry out a given function have decreased. In 1967, for example, the basic PDP8, perhaps the most widely used of DEC minicomputers, cost more than £7,000; nowadays the price of the PDP8A is little more than £1,000.

It would be a mistake, however, to think that most minicomputers are sold to individuals to use in their laboratories or offices. DEC estimates that 50–60% of its sales (by value) are not made to the final user but to another company which incorporates the computer into its own equipment, for example a mass spectrometer, where it will be used to control the operation of the equipment and process the data that emerges.

It would also be a mistake to imagine the minicomputer as simply a box that fits on to a table top and is accompanied by a typewriter input and output. That may have been the case in the late 1960s, but now a minicomputer is much more of a system, with the minicomputer proper accounting for relatively little of the cost compared with line printers, visual display units, graphics terminals, disk stores and the like.

In many ways the whole philosophy surrounding the manufacture of minicomputers distinguishes them from the larger machines. Not only do companies like Data General and DEC operate with far fewer of the trappings like prestige offices than many of the companies manufacturing mainframe computers, but the very nature of the minicomputer means that vast armies of engineers and service personnel are not required. One of the main attractions of minicomputers is

that they can be operated in the relatively dusty, humid and warm conditions of a normal laboratory or office, safe in the knowledge that they will not break down with the monotonous regularity of some mainframe machines. It is true that the criticism has been levelled against some companies manufacturing minicomputers that their after-sales service is not good, but on the whole minis seem to provide, pound for pound, much better value for money than the larger machines. One can understand why the manufacturers of big machines sometimes refer to minicomputing as 'underground computing'. It certainly gives them something to think about.

Minicomputers and scientists

Because minicomputers are tailored by their users to fit precisely the task required of them, it is difficult to generalise about the ways in which scientists use them. Almost any laboratory that requires data logging, control of equipment in real time or arithmetical calculations of any kind can profitably make use of a mini. If that mini can also be connected in to a large mainframe computer as a 'front-end machine', then the tasks

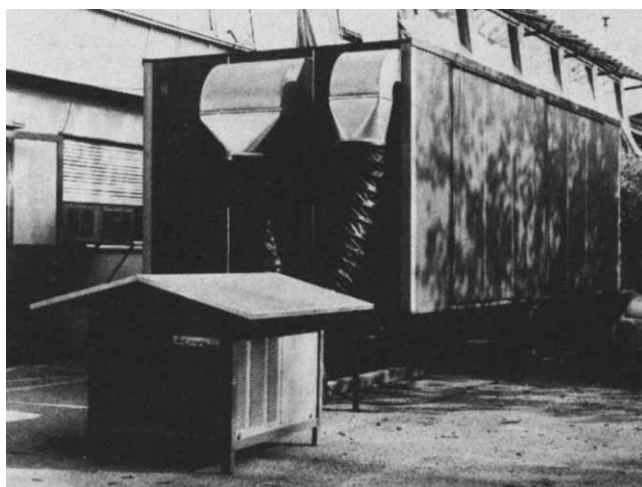


Fig. 1 This travelling computer laboratory developed at CERN contains three minicomputers, and can easily be moved from place to place.

which can be carried out (particularly 'number crunching') can be that much more sophisticated and complex.

A crystallographer, for example, might use a minicomputer system to provide views of a complex molecular structure from different angles whereas, an astronomer might incorporate a minicomputer into his telescope control equipment so that a given part of the sky can be tracked for long periods of time. Engineers, on the other hand, might well set up a mathematical model for a bridge—or an aircraft wing—and use the minicomputer to display on a visual display unit or graph plotter the consequences of certain input data (loading, sizes of beams, or whatever). Some people have found minicomputers a sufficiently attractive proposition to group them together into a travelling laboratory (Fig. 1).

The examples are so numerous that I shall describe in more detail the use made of minicomputers in a particular university department, the Department of Aeronautics at Imperial College, London. Typical is not the word to use, as no minicomputer system is typical, but certainly some of the characteristics are similar to some of those of other installations.

The department's minicomputing facilities certainly take up

rather more than a table top, a medium sized room in fact. They include the usual peripherals and in addition magnetic tape decks for data storage and the necessary equipment to link the department's facilities to the main college computer, a CDC machine. It is thus possible to collect, store and do calculations on data both locally and in the CDC machine as the need dictates.

The Aeronautics Department operates two hypersonic wind tunnels—one a 'gun tunnel' in which everything is over in 10 ms or so (but can be repeated about every 20 min) and the other a continuous low density nitrogen wind tunnel which requires accurate knowledge of flow rates, pressures and so on to establish the conditions of operation. Before the mini-computing facilities were acquired a year or so ago, the pressure profiles for the gun tunnel were recorded photographically using eight oscilloscopes and much of the subsequent data extraction was done manually. Now, however, the profiles are recorded and analysed by the minicomputer system, with the minimum of human intervention.

HELLO. THIS IS AN EXPERIMENT IN TAKING DETAILS OF MEDICAL SYMPTOMS BY A COMPUTER. IT IS DESIGNED TO HELP DOCTORS IN THEIR WORK, AND TO IMPROVE MEDICAL FACILITIES FOR THE PATIENTS AS WELL.

WE WOULD LIKE YOU TO HELP US WITH THIS AND IF YOU ARE PREPARED TO WOULD YOU PUSH THE BUTTON MARKED 'YES'. IF NOT PRESS THE BUTTON MARKED 'NO', AND IF YOU DON'T UNDERSTAND PRESS THE '?' BUTTON.

GO AHEAD AND PUSH ONE OF THE BUTTONS NOW? 0

GOOD, THANKS VERY MUCH. EVERYTHING THAT YOU SAY WILL BE TREATED IN THE STRICTEST CONFIDENCE AND ONLY DOCTORS AND NURSES WILL MAKE USE OF THE INFORMATION THAT YOU HAVE GIVEN. ARE YOU QUITE HAPPY ABOUT THIS? PUSH 'YES' IF YOU ARE, 'NO' IF YOU'RE NOT AND '?' IF YOU DON'T UNDERSTAND? 0

GOOD. THIS IS A COMPUTER TALKING TO YOU. I CAN ASK YOU QUESTIONS BUT I CAN ONLY UNDERSTAND SIMPLE ANSWERS, AND YOU GIVE ME THESE ANSWERS WHEN YOU PUSH ONE OF THE THREE BUTTONS IN FRONT OF YOU. THEREFORE ALL THE QUESTIONS CAN BE ANSWERED IN THIS WAY. DO YOU UNDERSTAND EVERYTHING I'VE SAID UP TO NOW? 0

BY THE WAY, IN YOUR JOB DO YOU EVER WORK AT NIGHTS? PRESS ONE OF THE BUTTONS NOW? 1

OK. NOW I WANT TO ASK YOU SOMETHING ABOUT YOUR CHEST. BEFORE YOU PUSH ONE OF THE BUTTONS I'D JUST LIKE TO REMIND YOU THAT IF YOU DON'T QUITE UNDERSTAND WHAT I MEAN BY THE QUESTIONS, THEN PRESS THE '?' BUTTON. TELL ME, DO YOU USUALLY COUGH FIRST THING IN THE MORNING IN THE WINTER? PUSH ONE OF THE BUTTONS NOW? 0

DO YOU USUALLY COUGH DURING THE DAY IN WINTER? PUSH ONE OF THE BUTTONS? 0

DO YOU COUGH LIKE THIS ON MOST DAYS FOR AS MUCH AS THREE MONTHS EACH YEAR? 6

OBVIOUSLY I CAN'T BE TOO PRECISE ABOUT THIS, BUT WOULD YOU SAY THAT YOU COUGH LIKE THIS ON MOST DAYS FOR AS MUCH AS THREE MONTHS IN A YEAR. PRESS YES OR NO THIS TIME? 1

WOULD YOU SAY THAT WHEN YOU GET A COLD IT GOES DOWN TO YOUR CHEST? 0

WHEN YOU GET A COLD, HOW LONG DOES IT TAKE TO CLEAR UP. FIRST, WOULD YOU SAY IT ONLY TOOK THREE OR FOUR DAYS? 1

IS IT ABOUT A WEEK? 0

Fig. 2 A conversation between a patient and a computer (from ref. 1). Replies: 1, no; 6, ?; 0, yes.

circuit and thus will be used to predict the performance of the completed circuit.

Medical applications

Minicomputers are also becoming of increasing importance in medicine—both in the diagnostic field, where they are slowly but surely reshaping the traditional doctor-patient relationship, and in the laboratory, where they are increasingly relieving medical technicians of time-consuming chores. For example, colorimetric tests carried out to detect the presence of given substances in body fluids using sequential multi-channel analysers can be speeded up and made more accurate by using a minicomputer. Not only can the data be produced in a form suitable for use on the wards—a transcription process previously left to the technicians—but the analysers can be automatically recalibrated against a standard solution every so often, and an automatic correction made to all measurements as the instrument slowly and inevitably drifts away from its calibrated state. The same goes for many other instruments used to make pathological measurements.

In the case of medical interviewing by computer, the aim is to get a patient to respond to a sequence of questions about his state of health which either gives the doctor sufficient information to make a diagnosis or establishes that the patient is for some reason unable to communicate with the computer (see, for example, Fig. 1). The keeping of medical records is a whole new area again in which minicomputers seem set to spark off a revolution. It is true that hospitals have tried keeping records on large central computers before, but some schemes have come unstuck because of a reluctance on the part of doctors to write up reports in long-hand and then, perhaps the next day, rewrite them into the computer. They rightly think that is a waste of time, but could undoubtedly be persuaded to use a system which was fully accessible to them the first and only time they write up their reports and observations.

Industry and energy

The capability of the minicomputer for process control clearly has many implications for manufacturing industry, particularly as the operation of manufacturing plant in sub-optimum conditions often means that energy is being wasted. The aluminium industry is just one that has benefited from minicomputer control, in that several companies use minis to ensure that the resistance of the electrolytic cells in which the alumina is reduced does not vary, and with it the current consumption. No foreman can hope to check the voltage drop across each cell and adjust the electrode positioning sufficiently quickly that the cells operate in non-optimum conditions for less than a few minutes, and the outcome, when multiplied up, is a larger consumption of energy than necessary. Plainly any industrial process which uses any kind of control loop can profitably be linked up to a 'dedicated' minicomputer, be it steel rolling or the production of chemicals.

Decreasing size

As microprocessors come into their own, the price of minicomputers is likely to continue to fall, with the bulk of the space inside a minicomputer being taken up with connections to and from a single chip. Microprocessors are, however, likely to find their greatest application as preprogrammed computers within other pieces of apparatus. Whatever else happens, underground computing is here to stay.

¹ Evans, C. R., Kinchin, C. G. J., Price, H. C., and Whittle, P. B., *NPL Report Com 73* (National Physical Laboratory, Teddington, 1974).

In the case of the continuous wind tunnel, it used to take up to two days to find the operating conditions by submitting data in the conventional way to the central college computer. Now they have the answer in 30 s.

As well as using the mini system for developing and testing mathematical models, for example of the behaviour of a helicopter rotor blade in various conditions, the department will also use it to control a milling machine and to develop electronic circuits. In the latter case, the computer will simulate the operation of each component that goes to make up a

articles

Diversity of cross-bridge configurations in invertebrate muscles

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X-ray diffraction patterns from relaxed invertebrate muscles reveal the thick filament symmetries and cross-bridge configurations. The cross bridges are substantially angled to the filament axes. The results on symmetry are generally consistent with Squire's model.

X-RAY diffraction studies of striated vertebrate and insect muscles^{1,2} have not yet established the symmetry of the array of myosin cross bridges on the thick filaments, nor the configurations of the resting cross bridges. Squire^{3,4} has proposed a scheme for thick filament structure that predicts a relationship between the rotational symmetry and the diameter of the filament backbone, and on this basis has suggested possible values for the rotational symmetries of vertebrate striated and insect muscle thick filaments. These proposals are supported by recent chemical evidence^{5,6}.

We describe here the detailed low angle X-ray diffraction patterns given by relaxed striated muscles from three invertebrate animals: *Limulus* (horseshoe crab), *Homarus* (lobster) and *Placopecten* (scallop). These patterns differ from those given by vertebrate and insect muscles in one important respect; the diffraction from myosin shows sampling from the hexagonal filament lattice only on the equator. In two cases, this enables us to determine the rotational symmetries from the reflection profiles; moreover, the relative intensities of the layer lines can be used in a straightforward way to determine the configurations of the resting cross bridges. The cross bridges seem to be markedly tilted in the resting state, and to take up diverse configurations and positions. Our conclusions on thick filament symmetry are broadly consistent with Squire's model⁴.

Description of diffraction patterns

We describe here only those features of the diffraction patterns that relate to thick filament symmetry and cross-bridge configuration. Other aspects of the patterns, and full details of their analysis, will be described elsewhere.

The pattern from *Limulus* muscle has been described by us previously⁷ (Fig. 1a); it is characterised by a series of strong myosin layer lines indexing on a repeat of $3 \times 146 = 438$ Å. The first and third are the strongest of these; the fourth is of moderate intensity; and the second, fifth and sixth layer lines are weak. The meridional reflection on the 146-Å (third order) layer line has a clear subsidiary maximum associated with it, 270 Å from the meridian.

In the pattern from *Homarus* muscle (Fig. 1b) the only layer lines which can be unambiguously assigned to myosin

are those at 146 and 73 Å. These have strong meridional peaks, and the 146-Å layer line has a clear subsidiary peak 230 Å from the meridian.

The *Placopecten* muscle gives the most detailed pattern of the three (Fig. 1c); the myosin layer lines are clear and index on a repeat of $10 \times 146 = 1,460$ Å, the first strong non-meridional layer line being at 487 Å (third order), with weaker ones at 208 Å (seventh order) and 112 Å (13th order). The 146 and 73-Å layer lines are remarkably detailed; at least two subsidiary maxima accompany the meridional peak in each case, falling at different positions on the two layer lines (270 and 145 Å off meridian for the 146-Å layer line and 365 and 165 Å for the 73-Å layer line). Additional meridional reflections occur at 98 and 58 Å, which index as the third and fifth orders of $2 \times 146 = 292$ Å; these have a different profile, with no apparent subsidiary maxima.

Model calculations

We have compared the observed diffraction patterns with those calculated for model thick filaments with different helical symmetries and cross-bridge configurations. We assumed that each bridge corresponds to a single myosin molecule, and that the diffraction from the tails of the molecules (forming the filament backbone) could be neglected. A cross bridge was modelled as either a single cylinder (150 Å long $\times$ 56 Å diameter), or as two cylinders (each 150 Å long $\times$ 40 Å diameter) representing the two subfragment-1 (S1) subunits.

Figure 2 illustrates some relevant concepts of helical symmetry. Sections of a single type of surface lattice may be rolled up to form different multi-stranded helices (Fig. 2a); these differ in rotational symmetry (order N) and diameter, but have a very similar local geometrical relationship between neighbouring lattice points. This relationship can be determined easily from the layer line spacings in the diffraction patterns, which give directly the angular relation of one layer of cross bridges to the next (compare definition of screw order in Fig. 2). The axial translation between layers of cross bridges is within 1 or 2% of 145 Å in all thick filaments. The crucial point is that the different helices give very similar diffraction patterns, in which the different diameters are manifest only in the detailed profiles of the reflections. Qualitative aspects of the intensity distribution among the layer lines can be understood by reference to the (n,l) -plot; alignment of cross-bridge density with a particular set of helical tracks produces enhancement of the corresponding layer lines, irrespective of the rotational symmetry (Fig. 2b and c).

The following points were established by our calculations of the cylindrically averaged intensity distribution⁸ expected from helical arrays of cross bridges.

(1) The 146-Å layer line has a strong meridional peak, with a weaker subsidiary peak. For models with cross-bridge lengths

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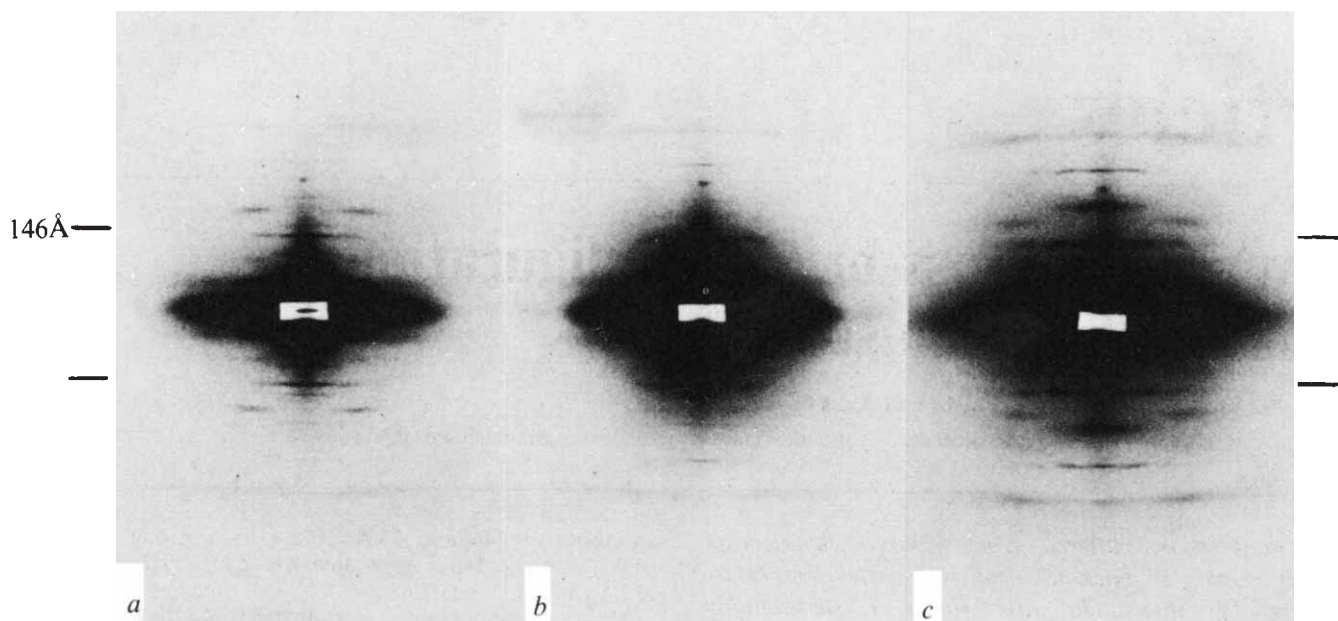


Fig. 1 Low angle X-ray diffraction patterns obtained from strips of muscle about 1 mm in diameter, mounted in a cell under tension at 4 °C. Glycerinated strips (prepared as before⁷) were immersed in a relaxing buffer containing ATP; living muscles were kept in an appropriate aerated Ringer solution. A mirror-monochromator X-ray camera with a specimen-to-film distance of 400 mm was used. The X-ray source was an Elliott GX6 rotating anode loaded at 600 W. Multiple packs of Ilford Industrial G or Kodak Industrex AA film were used to record the patterns; intensity data were obtained from them by scanning on a Joyce-Loebl microdensitometer or an Optronics rotating drum scanner. Background was subtracted using scans on either side of the layer line in question; for more detailed analysis of the profiles of meridional reflections and their subsidiary maxima, Optronics scans on 25 μ m or 100 μ m rasters allowed a similar subtraction of background to be made at each point along the layer line. Reflection profiles were further corrected by deconvoluting the profile of the main beam, assumed to be Gaussian. *a*, Pattern from abdominal muscle of *L. polyphemus* (see ref. 7). Glycerinated muscle in relaxing buffer (40 mM KCl, 8 mM MgCl₂, 1 mM EGTA, 5 mM NaATP, 10 mM histidine, 0.1 mM sulphadiazine, pH 7.0). *b*, Pattern from deep abdominal extensor muscle of *H. americanus* (see ref. 11). Glycerinated muscle in relaxing buffer. *c*, Pattern from striated (translucent) adductor muscle of *P. magellanicus*; living relaxed muscle in *Limulus* Ringer solution⁷.

up to about 200 Å, the distance of this subsidiary from the meridian depends on the radius at which the cross bridges are centred, and is only slightly affected by bridge configuration. Axial tilt (see Fig. 2) of the cross bridges weakens the meridional peak more than the subsidiary peak, so that the ratio of intensities of the two reflects the amount of tilt. The intervening minimum becomes less distinct when tilt is increased without azimuthal twisting.

(2) On the other (non-meridional) layer lines the radial positions of the maxima depend not only on bridge radius but also on rotational symmetry of the filament. The axial spacings of these layer lines are determined by the helical symmetry; the strongest layer line is that corresponding to the pitch of the myosin helix (*P* in Fig. 2*a*), and for a given symmetry and bridge radius its intensity and profile are remarkably insensitive to tilting and twisting of the cross bridges. Other layer lines are more sensitive to changes in bridge configuration, as expected from the (*n*,*l*)-plot (Fig. 2*b*).

(3) When the S1 subunits in each bridge are separated, additional effects are observed; the subsidiary maxima on the 146 and 73-Å layer lines are shifted in position, and the intensities of all the layer lines are further modulated by interference between the two subunits.

Interpretation of diffraction patterns

The spacings of the layer lines in the horseshoe crab pattern indicate that there are three layers of cross bridges separated by 146 Å in the repeat length of 438 Å (compare Fig. 2*a*). The profile of the 146-Å layer line shows that the cross bridges are centred at a radius of about 165 Å from the helix axis. The profiles of the other non-meridional layer lines are accounted for if there are three or four cross bridges at each level in the thick filament (compare Fig. 2*a*). The intensity of the 146-Å meridional is no greater than that of the first layer line at 438 Å, suggesting that the cross bridges are tilted axially, by at least 30° from the normal to the filament axis. The striking

modulation of the layer line intensities can be explained by a substantial degree of azimuthal twist of the cross bridges; combinations such as 60° twist and 30° tilt give a good fit for the profiles and intensities of layer lines 1–6 (Fig. 3*a*). With our present data, there is no need to invoke the separation of S1 subunits.

The *Homarus* pattern does not enable us to determine the symmetry of the surface lattice, but we find from the profile of the 146-Å layer line that the radius of the cross bridges from the axis is about 140 Å. The unusually small (3:1) ratio of meridional to off-meridional intensity on the 146-Å layer line indicates some axial tilt of the cross bridges. The closeness of the bridges to the axis suggests a reason for the absence of non-meridional layer lines: models with such a small radius and a high (for example, sixfold) rotational symmetry give very weak layer lines if the bridges are even slightly twisted azimuthally (compare Fig. 3*b*).

The spacings of the layer lines in the *Placopecten* pattern indicate that layers of cross bridges separated by 146 Å are related by a slightly different screw operation from that in *Limulus*: the ratio of the spacings of the strong 487 and 146-Å layer lines is here 10/3, so that the repeat length is $10 \times 146 \text{ Å} = 1,460 \text{ Å}$, although the angular relationship between 146-Å layers is only slightly different from that in *Limulus*. The screw order is defined in Fig. 2. The layer line profiles are more complex in this pattern than in the other two, but we find that the striking difference in profile between the 146 and 73-Å layer lines is well explained by certain specific bridge configurations. The bridges must have large axial extent when projected on to the axis, but the presence of clear minima in the profiles shows that this does not result from tilting single rods: instead, a good fit results when the two S1 subunits are separated axially, for example, by tilting about 20° up and 20° down, respectively. The profiles, interpreted in this way, indicate that the bridges are centred about 170 Å from the filament axis; the non-meridional layer lines can then be

fitted when the rotational symmetry is six- or sevenfold. Since the non-meridional layer lines are strong, azimuthal twisting of the bridges must be small (compare Fig. 3c). The presence of meridional reflections at odd orders of 292 Å in patterns from relaxed (but not rigor) muscles can best be explained by small axial perturbations of the bridges at alternate 146-Å levels along the filament (Millman and Bennett, unpublished).

In summary, the cross bridges in these muscles seem to lie at similar radii from the filament axes in spite of differences

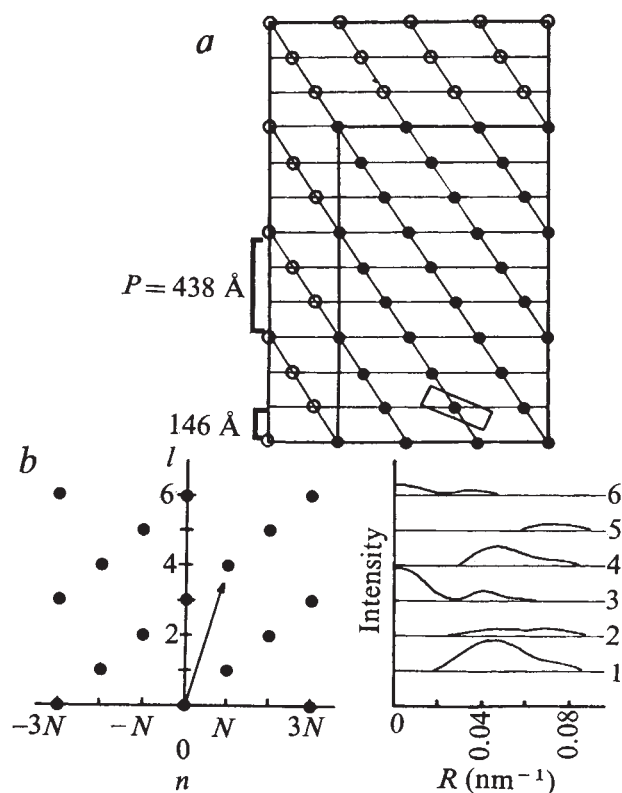


Fig. 2 *a*, Radial projections of two multi-stranded helices derived from the same surface lattice, but with differing rotational symmetries ($N = 3, 4$). The blocked area represents schematically the appearance in radial projection of a cross bridge tilted axially and azimuthally. We define axial tilt as the angle between the long axis of the bridge and the plane normal to the filament axis, and azimuthal twist as the angle (measured in this plane) between the bridge axis and the radius from the helix axis to the bridge's centre of mass. The two helices have symmetries S_3C_3 and S_3C_4 respectively, according to a notation suggested to us by D. L. D. Caspar: the notation $S\sigma C_N$ indicates combination of a screw axis $S\sigma$ (of screw order $\sigma = 2\pi/N\phi$ where ϕ is the azimuthal rotation between units at different levels) and a point group C_N (where N is the order of rotational symmetry). Note that σ is the number of units per turn of the helix divided by the rotational symmetry: thus helices with the same value of σ but different values of N have the same local geometry in the surface lattice. Moreover, the diffraction pattern from a helix gives the value of σ directly. Thick filaments of *Limulus* have either S_3C_3 or S_3C_4 symmetry (see text); vertebrate striated muscle filaments would be S_3C_2 (Huxley and Brown's 6₂-helix model¹), or S_3C_3 or S_3C_4 (Squire's models^{3,4}). In *Placoepecten* muscle, the layer line spacings in the diffraction pattern show that $\sigma = 10/3$ (see text); and the rotational symmetry is either six- or sevenfold: thus the filament has $S_{3,33}C_6$ or $S_{3,33}C_7$ symmetry on this notation. *b*, (n, l) -plot for the surface lattice in *(a)*, which describes the diffraction from any of the helices derived from it. The arrow indicates the direction in the diffraction pattern of the spike of intensity corresponding to the cross bridge orientation shown in *a*. Changes in orientation of this spike cause larger changes of intensity on layer lines far from the origin than on those closer in. *c*, Cylindrically averaged intensities calculated for the helix with $N = 3$, with single-rod cross bridges tilted 30° axially and 60° azimuthally (as in *a*). The weak second layer line, the strong fourth layer line, and the outward displacement of the peak on the fifth layer line are as expected from consideration of the (n, l) -plot.

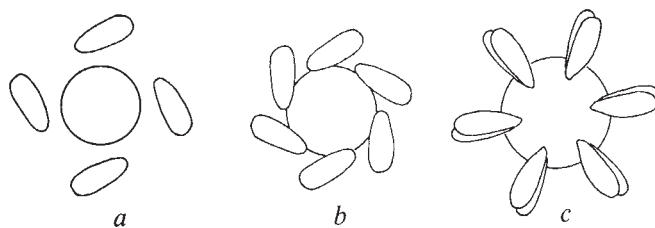


Fig. 3 Scheme illustrating the diversity of bridge location and configuration in myosin filaments: *a*, *Limulus*; *b*, *Homarus*; *c*, *Placoepecten*. The bridges at one level in each filament are shown, and the circle represents the backbone diameter as estimated from electron microscopy (see text references). Rotational symmetries for *Limulus* and *Placoepecten* are those which give agreement with present data; the symmetry for *Homarus* is hypothetical (see text).

in screw and rotational symmetries. The orientations of the cross bridges vary: *Limulus* bridges, viewed as single rods, are tilted axially and twisted azimuthally; in *Homarus* they are also tilted axially, and may be slightly twisted azimuthally; and in *Placoepecten* muscle there is evidence for axial splaying of the S1 subunits, with little or no azimuthal twist, and a periodic perturbation of the bridges at alternate levels.

Implications of the patterns

These are the first X-ray diffraction patterns that reveal the rotational symmetries of the thick filaments: three- or fourfold in *Limulus* muscles and six- or sevenfold in *Placoepecten* muscles. Squire's model predicts a relationship between the rotational symmetry and the diameter of the filament backbone, but we have not been able to determine this diameter directly from the X-ray patterns because of limitations of the equatorial data. Electron microscope studies suggest that thick filaments of *Limulus* are about 195 Å in diameter¹⁰ whereas filaments of *Homarus* and *Placoepecten* may be larger (210 and 225 Å, respectively^{11,12}). Until we obtain more precise values for the backbone diameters in these muscles, we cannot test Squire's model rigorously; but our results on symmetry, together with the values for diameters obtained by electron microscopy, seem to be consistent with his proposal on the structure of thick filament backbones.

Our results emphasise that although there seems to be a close relationship between the backbone structures in the thick filaments of different muscles, the cross bridges adopt various configurations. One similarity, however, is that in all these muscles the relaxed cross bridges are tilted away from the normal to the filament axis. If the bridges are attached to actin in a right-angled configuration before the power stroke of the contractile cycle, then some angular movement of the bridges must occur on activation or initial attachment in these muscles.

Our results on cross-bridge configuration depend on assumptions about the dimensions of the cross bridges. We have used those dimensions found for vertebrate S1 attached to actin by three-dimensional reconstruction from electron microscope images¹³. The bridges in our three relaxed muscles may depart from these dimensions, but must be elongated: we found no way of explaining the diversity of patterns other than in terms of specific orientations of rod-shaped bridges. As more precise information becomes available, our solutions will have to be revised accordingly, but we believe that the models (Fig. 3) illustrate the variety of bridge configurations required to account for the X-ray observations.

The diffraction patterns from relaxed vertebrate and insect muscles^{1,2} contain, in principle, similar information on filament symmetry and cross-bridge configuration, but the presence of sampling necessitates the use of correction factors for the meridional intensities and prevents observation of the full layer-line profiles. Sampling in the pattern from relaxed frog muscle is reduced when the muscle is stretched to a length where thick and thin filaments no longer overlap¹⁴; the fourth

layer line is then observed to be prominent, as in the pattern from relaxed *Limulus* muscle, suggesting that similar tilting and twisting of the bridges occur in this muscle. Further evidence for tilting is provided by the absence of a clear minimum on the 143-Å layer line. Squire¹⁵ reports that analysis of the sampled pattern leads to a similar conclusion.

The three invertebrate muscles we have studied possess three different regulatory mechanisms (actin-linked, myosin-linked, and double regulation¹⁶). We are now using X-ray diffraction to determine how these regulatory systems influence attachment of the cross bridges to actin.

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³¹P NMR studies of NADPH and NADP⁺ binding to *L. casei* dihydrofolate reductase

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The ³¹P NMR spectra of NADPH and NADP⁺ in their complexes with dihydrofolate reductase show effects from three-bond ¹H-³¹P coupling constants which indicate that the conformation about one of the C₅'-O₅' bonds changes when the coenzyme is bound to the enzyme. ³¹P chemical shifts demonstrate that the 2'-phosphate group is in the dianionic state in the complex.

DIHYDROFOLATE reductase catalyses the NADPH-linked reduction of dihydrofolate to tetrahydrofolate. As part of a wider study of the binding of ligands to the dihydrofolate reductase from *Lactobacillus casei*, we have used high resolution nuclear magnetic resonance (NMR) spectroscopy to study the complexes of NADPH and NADP⁺ with the enzyme^{1,2}. These experiments showed that both coenzymes bind tightly to the enzyme, with a stoichiometry of 1 mol per mol enzyme, and provided some evidence that a change in the conformation of the enzyme accompanies coenzyme binding.

In ¹H NMR studies of the complexes of tightly bound ligands, it is often difficult to distinguish the resonances of the bound ligand from the complicated spectrum of the protein. This problem can be circumvented by selective deuteration, or by studying nuclei other than protons. We have used ¹³C NMR to study the binding to dihydrofolate reductase of NADP⁺ enriched to 90% with ¹³C at the carboxamido carbon on the nicotinamide ring². A logical extension of this kind of experiment is to monitor the ³¹P resonance signals of the coenzyme. ³¹P is the naturally occurring isotope of phosphorus, and is relatively sensitive to NMR detection. Several other workers have used ³¹P NMR to study ligand binding to macromolecules^{3–9}, and Houlst *et al.* used this technique to study phosphorylated metabolites in muscle¹⁰. We have begun our ³¹P NMR studies of coenzyme binding to dihydrofolate reductase by examining the binary complexes of the enzyme with NADPH and NADP⁺, and the results of these experiments are reported here.

³¹P chemical shift measurements

The ¹H noise-decoupled ³¹P NMR spectrum of NADPH

(Fig. 1a) consists of two absorption bands in the intensity ratio 1:2. The low field signal (0.47 p.p.m.) arises from the 2'-phosphate group, and that at -13.78 p.p.m. arises from the two phosphorus nuclei in the pyrophosphate group, which accidentally have the same chemical shift¹¹. In the binary complex of NADPH with dihydrofolate reductase (Fig. 1b) a markedly different spectrum is observed. The 2'-phosphate resonance is shifted downfield by 2.19 p.p.m., whereas the resonances of the two pyrophosphate phosphorus nuclei are both shifted upfield, but to very different extents (0.16 and 2.65 p.p.m.). Since these latter two phosphorus nuclei are now magnetically non-equivalent, their signals appear as an AB quartet, with ²J_{P-P} = 20.8 Hz. The small intensity difference between the two halves of the quartet is almost certainly due to a small difference in relaxation time. At present, it is not possible to assign the two pyrophosphate ³¹P NMR signals in the complex to the two individual phosphorus nuclei. Further addition of NADPH to give a molar ratio of NADPH to enzyme of 2:1 led to the appearance of additional resonances at the positions of those of free NADPH, with no other changes in the spectrum (not shown). Clearly there is only slow exchange (on the NMR time scale) between free and bound NADPH.

Similar experiments were carried out with NADP⁺. In the binary complex of NADP⁺ and dihydrofolate reductase, the chemical shift of the 2'-phosphate resonance is identical to that found for the NADPH complex (though the ³¹P chemical shifts of this group are different in free NADP⁺ and NADPH, see below). Again, both pyrophosphate signals are shifted upfield to different extents (there is a small non-equivalence of the two pyrophosphate phosphorus nuclei in free NADP⁺; see also ref. 11). The magnitude of the larger of the two shifts is, however, distinctly less than that seen with NADPH. The chemical shifts are summarised in Table 1. The ³¹P signals from the enzyme-NADP⁺ complex are somewhat broader than those from the enzyme-NADPH complex. This is probably due to an exchange contribution to the linewidth in the former complex, as observed in earlier ¹³C studies².

In the free coenzymes, the 2'-phosphate group has a pK of 6.1 for NADPH and 6.4 for NADP⁺. The ³¹P resonance from this group shows a corresponding change in chemical shift with pH (see also ref. 11), the total titration shift being 3.9 p.p.m. (Fig. 2). In contrast, in the complexes with the enzyme the

2'-phosphate resonance of both NADPH and NADP⁺ was independent of pH within the range pH 4.5 to 7.5, occurring at a position about 1.7 p.p.m. downfield of the position of the dianionic form of the 2'-phosphate in the free coenzymes. The chemical shifts and $^2J_{P-O-P}$ coupling constant of the pyrophosphate group were also independent of pH over the same range.

³¹P-¹H spin-spin coupling constants

The remarkably narrow resonance lines ($\Delta\nu_{1/2} \sim 7$ Hz) observed in the ¹H noise-decoupled ³¹P spectra of the NADPH-enzyme complex encouraged us to examine the single-resonance ³¹P spectrum. In the single-resonance ³¹P spectrum of free NADPH

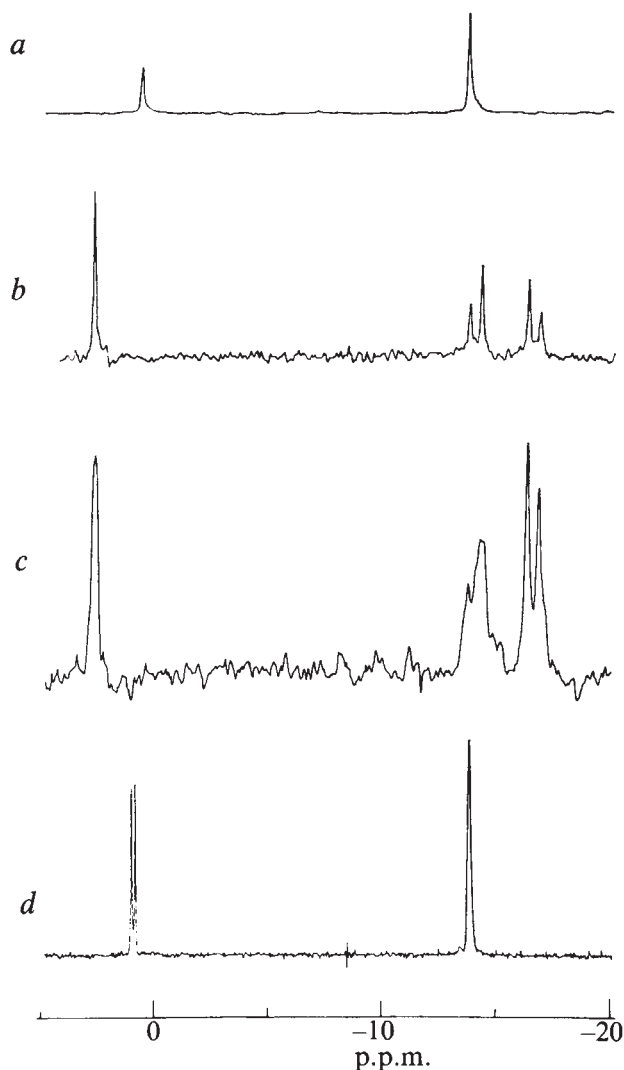


Fig. 1 *a*, The ³¹P proton noise decoupled spectrum of 10 mM NADPH at pH 6.9 (1,000 transients, 0.5 s acquisition time). *b*, The ³¹P proton noise decoupled spectrum of 1.3 mM NADPH in the presence of 1.3 mM *L. casei* dihydrofolate reductase at pH 6.9 (100 blocks of 1,000 transients 0.5 s acquisition time). *c*, ³¹P single resonance spectrum of 1.1 mM NADPH in the presence of 1.1 mM *L. casei* dihydrofolate reductase at pH 6.9 (319 blocks of 1,000 transients 0.5 s acquisition time). *d*, ³¹P single resonance spectrum of 50 mM NADPH at pH 7.9 (1,777 transients, 2.0 s acquisition time). All samples were dissolved in a D₂O buffer solution containing 500 mM KCl and 15 mM *bis*-Tris in the presence of 1 mM EDTA (except for sample used for spectrum *d*) which contained 15 mM EDTA). The ³¹P Fourier transformed spectra were obtained at 40.5 MHz using a spectrometer (Varian XL-100) equipped with a computer (VDM 620 i). Exponential weighting functions of 0.1 s (spectrum *c*) and 0.2 s (spectra *a* and *b*) were used. The chemical shifts were externally referenced to 50 mM K₂HPO₄ solution (pH 8.0) contained in a capillary. The sample temperature was maintained at 11 ± 1 °C using a temperature unit V 4343.

Table 1 ³¹P chemical shifts of NADPH and NADP⁺ and their complexes with dihydrofolate reductase

	2'-Phosphate	Pyrophosphate	
		A	B
NADPH (pH 6.9)	0.47		-13.78
NADPH-enzyme	2.66	-13.94	-16.47
NADP ⁺ (pH 6.9)	-0.22		(-14.47)
			(-14.15)
NADP ⁺ -enzyme	2.72	-14.32	-16.23

The chemical shifts are in p.p.m. and were externally referenced to 50 mM K₂HPO₄ solution (pH 8.0) contained in a capillary. Positive shifts to low field.

(Fig. 1*d*) the 2'-phosphate resonance shows a doublet splitting ($^3J_{P-O-C_2'-H} = 6.7$ Hz). No splittings can be resolved on the pyrophosphate signal, but from the line-broadening the couplings to the 5' protons must be small ($^3J_{P-O-C_5'-H_A} + ^3J_{P-O-C_5'-H_B} < 6$ Hz) (see also ref. 11).

The single-resonance ³¹P spectrum of the NADPH-enzyme complex (Fig. 1) suggests that the two phosphorus nuclei in the pyrophosphate are coupling to different extents to their corresponding 5' protons in the bound state. If reliable three-bond coupling constants could be measured this would enable us to determine the conformation about the C_{5'}-O_{5'} bonds in the bound coenzyme. Smith *et al.*¹² and others¹³⁻¹⁵, investigated the dependence of ³¹P-O-C-H coupling constants on the dihedral angle, θ , about the central bond. They found that there is a large difference in the magnitude of $^3J_{P-O-C-H}$ between *gauche* ($^3J_{P-O-C-H} = 1.8$ Hz) and *trans* ($^3J_{P-O-C-H} = 20.6$ Hz) conformations.

Unfortunately, the difference in linewidths between the ¹H noise-decoupled and single-resonance ³¹P spectra cannot be used directly to provide estimates of the coupling constants. A factor which must be considered in the measurement of coupling constants in large molecules is the possibility that rapid relaxation of one of the coupled nuclei is influencing the observed shape of the multiplets. Thus if the 5'CH₂ protons are rapidly relaxed then the measured coupling constants to the ³¹P nucleus could be smaller than the true coupling constants. The 5'CH₂ proton resonances cannot be observed in the ¹H spectrum of the coenzyme-enzyme complex which prevents us from measuring their *T*₂ values. By assuming a reasonable value for the correlation time of the complex, however, we have estimated that the *T*₂ values are in the range 0.02-0.03 s. Because of the dominant intramolecular contribution to the relaxation it is unlikely that the *T*₂ values for the two pairs of 5'CH₂ protons will be very different. We have calculated the effects of such relaxation rates on spin-multiplets with coupling constants in the range 1-20 Hz using the theory for the effects of exchange on line-shapes^{16,17}. Thus for the case of a doublet arising from coupling to such a relaxed proton when the coupling constant is 5 Hz the observed line broadening is ~ 1 Hz and for a coupling constant of 20 Hz the line broadening is ~ 16 Hz. For the high field pyrophosphate, the fact that we observe very little line broadening in the single resonance spectrum indicates that both P-O-C-H coupling constants are small (< 5 Hz); this of course assumes that our estimate that *T*₂ ≥ 0.02 s is correct. The lower field pyrophosphate shows considerable line broadening (~ 13 Hz) indicating that the sum of the coupling constants must be greater than 13 Hz (the existence of such large broadening shows that for these 5'CH₂ protons *T*₂ ≥ 0.02 s).

Ionisation state and conformation

The observation that the chemical shift of the 2'-phosphate ³¹P resonance is identical in the NADPH- and NADP⁺-reductase complexes clearly indicates that the 2'-phosphate group is binding in the same ionisation state and to the same site on the enzyme in both complexes. Since the chemical shift of this resonance is independent of pH within the range 4.5 to 7.5 in the complexes, the *pK* of the 2'-phosphate group must be

at least three units different from its value in the free coenzyme. The position of the resonance (1.7 p.p.m. downfield from the dianionic and 4.5 p.p.m. downfield from the monoanionic form of the free coenzyme) suggests that the 2'-phosphate group binds in the dianionic form. This is confirmed by studies of the binding of 2'-AMP (B.B., J.F. and G.C.K.R., unpublished); for this compound the bound ^{31}P chemical shift is identical to that for the coenzyme complexes, and the binding constant decreases by more than a factor of ten on decreasing the pH from 7.0 to 5.5.

The decrease in pK of the 2'-phosphate group of the coenzyme by more than three pH units on binding to dihydrofolate reductase suggests that an electrostatic interaction between the phosphate group and a cationic group or groups makes a major contribution to the binding energy. Perhaps the most likely explanation of the additional downfield shift (1.7 p.p.m.) of the ^{31}P resonance from that expected for the dianionic ionisation state is that it is a linear electric field effect resulting from the proximity of a positively charged group on the enzyme. This interaction of the 2'-phosphate group presumably makes a major contribution to the hundred-

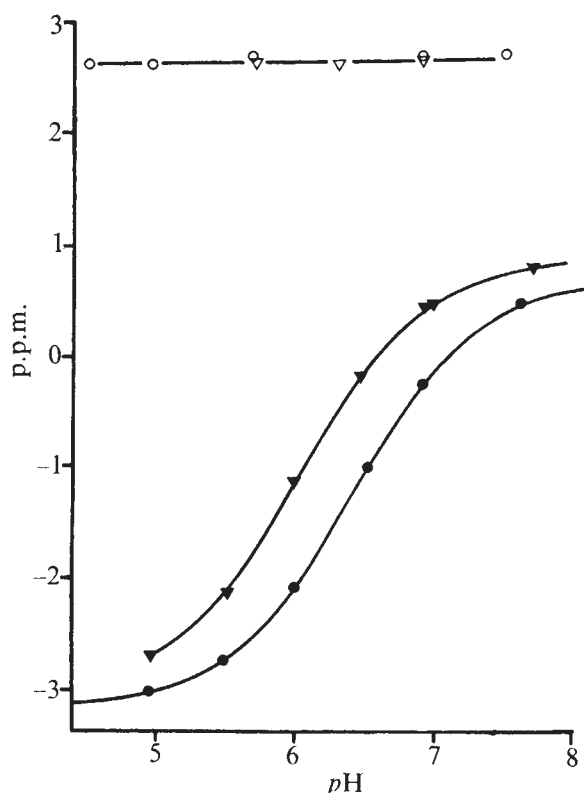


Fig. 2 Variation of chemical shift of the 2' phosphate ^{31}P resonance of NADP^+ (●, ○) and NADPH (▼, ▽) with pH. The open symbols refer to the coenzyme bound to dihydrofolate reductase and the solid symbols to the free coenzymes. The pH values are uncorrected meter readings (glass electrode) from D_2O solutions.

fold difference in the K_m values for NADPH and NADH seen for dihydrofolate reductase (J. Dann and G.C.K.R., unpublished).

The pattern of chemical shift changes for the two ^{31}P resonances of the pyrophosphate group is similar for both NADPH and NADP^+ complexes. One of the resonances is only slightly shifted in both cases; this and its $^3J_{\text{P-O-C-H}}$ coupling constants, indicate that this resonance represents the same phosphorus nucleus in both complexes. The other ^{31}P resonance shows a large upfield shift, which is significantly greater in the NADPH -enzyme complex than in the NADP^+ -enzyme complex. It is difficult to assess the significance of this difference, since we do not know the origin of the upfield shift on binding; it probably arises from a combination of

electric field effects and charge redistribution (perhaps accompanied by small changes in P-O-P angle¹⁸). ^1H NMR studies (our unpublished work) suggest that there may be conformational differences in the protein between the NADPH and NADP^+ complexes, but we cannot exclude the possibility that the difference in ^{31}P chemical shift is in some way a direct consequence of the positive charge on the nicotinamide ring of NADP^+ .

The narrow lines in the ^{31}P spectra of the coenzyme-dihydrofolate reductase complexes enabled us to measure $^{31}\text{P-O-}^{31}\text{P}$ spin-coupling and to observe the effects of $^{31}\text{P-O-C-}^1\text{H}$ spin-coupling. In experiments on other systems^{19,20}, coupling constants in the bound state were estimated by extrapolation for weakly binding ligands. The experiments described here, however, represent the first case in which direct coupling constant measurements have been possible for a ligand-protein complex.

The phosphorus nucleus giving rise to the highest-field signal shows small $^{31}\text{P-O-C-}^1\text{H}$ coupling constants (< 5 Hz), consistent only with a conformation in which it is *gauche* to both 5' protons. This is the conformation which is favoured for 5'-nucleotides²¹ and NADPH ¹¹ in solution. The other pyrophosphate phosphorus nucleus, which shows the smaller shift, shows a larger change in conformation differing from the *gauche-gauche* by at least 50° (based on considerations of the Karplus equation of Smith *et al.*¹²).

The conformations of NAD bound to lactate dehydrogenase²² and of ADP-ribose bound to alcohol dehydrogenase²³ are known from crystallographic studies. In both cases, the conformation about both $\text{C}_5'\text{-O}_5'$ bonds is *gauche-gauche*. For these enzymes, therefore, in contrast to dihydrofolate reductase, the binding of coenzyme is not accompanied by any change in the conformation about the $\text{C}_5'\text{-O}_5'$ bonds. At present, we are not able to specify which of the two $\text{C}_5'\text{-O}_5'$ bonds of NADPH shows a change in conformation on binding to dihydrofolate reductase, since we cannot yet assign the two pyrophosphate ^{31}P resonances in the complex. In view of the structural similarities in the coenzyme binding sites of a number of dehydrogenases^{24,25}, a comparison of the conformation of the bound coenzyme in other dehydrogenases will be of considerable interest, and we are attempting to extend our ^{31}P NMR studies in this direction, as well as studying the effects of substrate and inhibitor binding to dihydrofolate reductase on coenzyme conformation.

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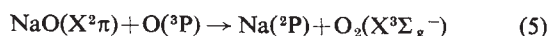
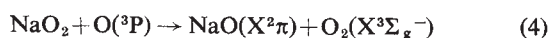
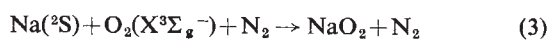
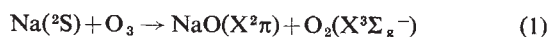
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letters to nature

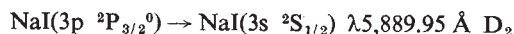
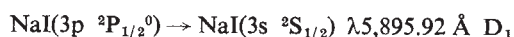
Sodium emission from long enduring meteor trains

ONE of the more perplexing atmospheric emission phenomena is the occurrence of long enduring visual meteor trains which, on rare occasions, are observed to persist for about 1 h. The mechanism responsible for the long lived luminosity is, however, elusive. Chapman¹ suggested that a source of night-time train luminosity is to be found in the store of recombination energy of free atmospheric atomic oxygen. He envisaged the role of Na atoms as continuously catalysing the transformation of atomic oxygen to molecular oxygen by sodium monoxide (NaO) formation so that the dissociation energy of O₂ was converted into sodium light with the aid of the atmospheric species O₃ and O₂. Here I examine the energetics of the sodium process and estimate the associated train luminosity.

The complete sequence of reactions is,



where the specific transitions are



At meteoric heights the atom interchange of NaO and O₃ to form NaO₂ or Na will be negligible.

When Chapman made his proposal the energetics of the various processes were not accurately known and, in particular, the exothermicity of reaction (5) was in doubt. Uncertainties arose because of the difficulties of measuring the dissociation energy, $D_0^\circ(\text{NaO})$, of the NaO molecule in the laboratory. Since the exothermicity of reaction (5) for producing the ground state sodium atom, Na(²S), is given by $D_0^\circ(\text{O}_2) - D_0^\circ(\text{NaO})$, then with $D_0^\circ(\text{O}_2) = 5.11 \text{ eV}$ the bond energy of NaO for the production of excited Na(²P) (2.10 eV) must be $D_0^\circ(\text{NaO}) < 66.2 \text{ kcalorie mol}^{-1}$ (3.0 eV). Although early studies indicated a lower limit to $D_0^\circ(\text{NaO})$ of 72 kcalorie mol⁻¹, theoretical and experimental work² has confirmed the value $60.3 \pm 4.0 \text{ kcalorie mol}^{-1}$, corresponding to $2.60 \pm 0.2 \text{ eV}$, making reaction (5) exothermic by $0.4 \pm 0.2 \text{ eV}$. The branching ratio of reaction (5) to yield ground state Na(²S) or excited Na(²P) is unknown.

Using the model that a solid meteoroid of mass ~30 g and velocity 40 km s⁻¹ will produce a meteor with visual magnitude of about -5, published ablation data³ can be extrapolated such that at zenith angle 30° the meteoroid will deposit meteoric atoms of linear density ~4 × 10¹⁷ cm⁻¹. With an

atomic abundance for Na of 0.5% (ref. 4) the line density of deposited Na atoms becomes $a_{\text{Na}} = 2 \times 10^{15} \text{ cm}^{-1}$. The fraction of these lost in forming Na⁺ by collisional ionisation is small at the velocity of meteors.

Extensive observational data show that the heights of long enduring visual trains are confined to 85–90 km. With a molecular diffusion coefficient at the lower height for Na atoms of 4.2 m² s⁻¹ and a radius, r , at time t of the radially expanding Na column given by $r^2 = 4Dt$ such a train will remain unresolved in a transverse direction by the naked eye for about 20 min. In such circumstances in the absence of turbulence and for an optically thin medium, the light intensity I is simply the sum of the quanta from all excited Na atoms in a cross section of the meteor train of unit length. Not only will atmospheric oxygen of number density [O_a] take part in the various reactions but also meteoric atomic oxygen of density [O_m]. With a meteoroid oxygen abundance of about 50%, then for a -5 mag meteor $a_{\text{O}} = 2.5 \times 10^{17} \text{ cm}^{-1}$. For ease of computation let all species have a common diffusion coefficient and treat atmospheric oxygen as a sink, then the set of equations describing the number densities of species may be written (representing, for convenience, number densities simply by the species symbol)

$$\frac{\partial}{\partial t} \text{Na} = D\nabla^2 \text{Na} + k_5 \text{NaO}(\text{O}_a + \text{O}_m) - k_1 \text{Na} \cdot \text{O}_3 - k_3 \text{Na} \cdot \text{O}_2 \cdot \text{N}_2 - k_2 \text{Na} \cdot (\text{O}_a + \text{O}_m) \cdot \text{N}_2$$

$$\frac{\partial}{\partial t} \text{NaO} = D\nabla^2 \text{NaO} + k_1 \text{Na} \cdot \text{O}_3 + k_2 \text{Na} \cdot (\text{O}_a + \text{O}_m) \cdot \text{N}_2 + k_4 \text{NaO}_2 \cdot (\text{O}_a + \text{O}_m) - k_5 \text{NaO} \cdot (\text{O}_a + \text{O}_m)$$

$$\frac{\partial}{\partial t} \text{NaO}_2 = D\nabla^2 \text{NaO}_2 - k_3 \text{Na} \cdot \text{O}_2 \cdot \text{N}_2 - k_4 \text{NaO}_2 \cdot (\text{O}_a + \text{O}_m)$$

$$\frac{\partial}{\partial t} \text{O}_m = D\nabla^2 \text{O}_m - k_2 \text{Na} \cdot \text{O}_m \cdot \text{N}_2 - k_4 \text{NaO}_2 \cdot \text{O}_m - k_5 \text{NaO} \cdot \text{O}_m$$

$$I = \Sigma d/dt [\text{Na}(^2\text{P})] = \Sigma k_5 \cdot f \cdot \text{NaO} \cdot (\text{O}_a + \text{O}_m) \text{ photons s}^{-1} \text{ cm}^{-1}$$

where ∇^2 is the Laplacian operator in cylindrical coordinates, and an initial meteor train radius of 1 m is assumed. Such a set may be solved using an implicit finite difference scheme⁵. Na atoms are continuously cycled and no overall night-time chemical loss of Na is included. The loss-lifetime from Na⁺ formation due to charge exchange with atmospheric O₂⁺ and NO⁺ will be of the order of 10⁶ s. Some initial charge transfer of Na atoms with ions of atoms with higher ionisation potential (such as Si, Fe, Mg) might be expected. Since the initial metal ion abundances are, however, generally rather less than those of Na atoms, no significant loss will occur.

Of the reactions (1)–(5) only the three-body association (reaction (3)) seems to have been studied in the laboratory and thus is the only one for which direct measurements of rate coefficient are available. For $T = 200 \text{ K}$, and height = 85–90 km we adopt (from ref. 6) $k_3 = 2.0 \times 10^{-32} \text{ cm}^6 \text{ s}^{-1}$. Rate coefficients which appear in the literature for the other reactions are based on the values for similar processes involving N and H and we use⁶ the following; $k_1 = 10^{-13} \text{ cm}^3 \text{ s}^{-1}$, $k_2 = 10^{-33} \text{ cm}^6 \text{ s}^{-1}$, $k_4 = k_5 = 10^{-11} \text{ cm}^3 \text{ s}^{-1}$. Uncertainties of a factor of three might be expected in all rate coefficients. The rate at which

the whole chemical cycle proceeds is determined mainly by reactions (1) and (3) and therefore in part by the concentration of the minor atmospheric species, O_3 . Using the first direct mass spectrometer measurements⁷ (obtained during early evening) we used densities of 8×10^8 and $3 \times 10^8 \text{ cm}^{-3}$ at 85 and 90 km respectively. The night-time densities of oxygen used are $3 \times 10^{11} \text{ cm}^{-3}$ at 90 km, and $7 \times 10^{10} \text{ cm}^{-3}$ at 85 km. The main gas densities (cm^{-3}) used are $[N_2] = 1.2 \times 10^{14}$ and $5.0 \times 10^{13} \text{ cm}^{-3}$ at 85 and 90 km, and $[O_2] = 3.2 \times 10^{13}$ and $1.3 \times 10^{13} \text{ cm}^{-3}$ at 85 and 90 km respectively. Solutions of the set of diffusion equations were computed (see for example Fig. 1). The emission for $t < 0.1 \text{ s}$ is caused by the effect of the large initial meteoric O_m concentrations and the associated emission decay by the subsequent rapid diffusion of O_m . For times up to

than this limit is a factor of 13 smaller than the observed frequency of trains of duration in excess of 30 min. A large proportion of trains reported⁹, however, occurred during major showers particularly the Perseids and Leonids when the bright meteor flux is several times larger than the sporadic flux.

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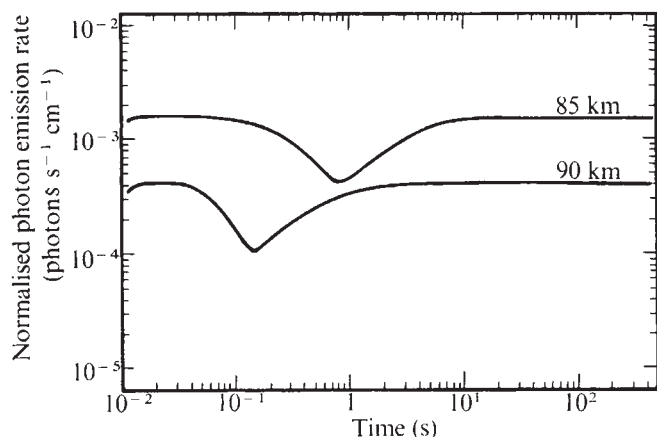


Fig 1 Solutions for the case of a meteor of approximately -5 mag occurring at heights of 85 and 90 km. Branching factor $f = 0.2$. Ordinate is the photon emission rate, I , normalised to the value of $4 \times 10^{13} \text{ photons s}^{-1} \text{ cm}^{-1}$. Parameters used are: $\alpha_{Na} = 2 \times 10^{15} \text{ cm}^{-1}$, $\alpha_{Om} = 2.5 \times 10^{17} \text{ cm}^{-1}$, $D_{85} = 4.2 \text{ m}^2 \text{ s}^{-1}$, $D_{90} = 10 \text{ m}^2 \text{ s}^{-1}$. An initial neutral atom radius of 1 m assumed at both heights.

several hundred seconds while the train remains unresolved the computed emission rates are 6.4×10^{10} and $1.7 \times 10^{10} \text{ photons s}^{-1} \text{ cm}^{-1}$ at 85 and 90 km respectively. The first point is how this emission compares with the observed train luminosities. Taking the estimate⁸ of the minimum detectable emission for the naked eye as $30 \text{ erg s}^{-1} \text{ cm}^{-1}$ and assuming that a clearly visible train is 2 mag brighter than this limit, the required emission rate in the yellow is $\sim 5 \times 10^{13} \text{ photons s}^{-1} \text{ cm}^{-1}$, indicating that the computed emissions for a -5 mag meteor fail by 7.0 and 8.3 mag. We infer that meteors of -12.0 and -13.3 mag could produce the required luminosity for 85 and 90 km respectively. The second important point is how the frequency of occurrence of such bright meteors compares with that of long duration trains. Since about 1 in 10^3 visual meteors produces a train of duration exceeding 10 s and with the observation that of those night-time trains of durations greater than 10 s in the extensive records of Olivier⁹ 1.5% had durations in excess of 30 min, then, with an observed visual rate of about 10 h^{-1} , it is inferred that the frequency of occurrence of trains longer than 30 min for a single observer is about $1.5 \times 10^{-5} \text{ h}^{-1}$. In comparison, the observed integrated flux¹⁰ of bright meteors indicates that the occurrence frequencies of meteors brighter than -12.0 and -13.3 for a single observer are 1.2×10^{-7} and $2.2 \times 10^{-8} \text{ h}^{-1}$ respectively.

If every sodium oxide reduction reaction produces an Na atom in an excited 2P state ($f = 1.0$) then at 85 km a meteor of about -10.25 mag is required to yield sufficient train luminosity. Though such bright objects certainly do produce long enduring trains⁹ the occurrence frequency of meteors brighter

Observations of eight globular clusters at 2.3 and 4.7 μm

THE Cerro Tololo infrared photometer¹ has been used to search several globular clusters for infrared emission between 2.3 and $20 \mu\text{m}$, principally to seek evidence for the presence of dust. Recently, more extensive observations using improved short wavelength instrumentation have been obtained at 2.3 and $4.7 \mu\text{m}$. The latter observations were concentrated on two groups of predominantly southern clusters: (1) two globular clusters (NGC 6388 and 6441) rich in metals and suggested² to be of particular interest for the detection of gas thought to be lost from stars during the normal course of stellar evolution; and (2) six clusters with short core relaxation times and large central densities discussed³ in connection with the possible presence of massive central black holes in clusters associated with X-ray sources.

The observations at 2.3 and $4.7 \mu\text{m}$ (with bandwidths of 0.37 and $0.60 \mu\text{m}$, respectively) discussed here were obtained on two nights in June, 1975 using the 1.5-m telescope on Cerro Tololo and an InSb detector on the dual-beamed photometer which operates at 10 Hz with an 80% duty cycle. Each cluster was carefully centred visually in the $10''$ aperture, after which precalculated offsets to the edge of the cluster were used to fix the starting point for a series of diametric drift scans. Typically, 40 drift scans in right ascension per cluster at each wavelength were obtained. As the fixed separation of $28.5''$ on the sky between the two beams (normally the 'star' and 'sky' positions) of the photometer was less than the cluster diameter, the drift scan technique actually measures an intensity gradient, and data reduction was performed on this basis. The integration time was set to equal the time to drift the beam separation distance; each drift scan therefore can be thought of as a number of discrete steps.

Table 1 lists the clusters observed, the number of scans obtained at each wavelength, the steps per scan, the total integration time per step, and the approximate apparent magnitude of each object. As each spatially resolvable point was effectively observed for only a relatively short time ($\sim 100 \text{ s}$), the detection threshold of the measurements is not as low as could be achieved by observing a single cluster-sky-point pair for a longer time. But, as one of the largest uncertainties in the theoretical^{2,3} and observational (refs 4 and 5 and M. G. Smith, J.E.H. and S. J. Shawl, unpublished) work done to date on the presence of gas and dust in globular clusters relates to the volume over which one might expect to

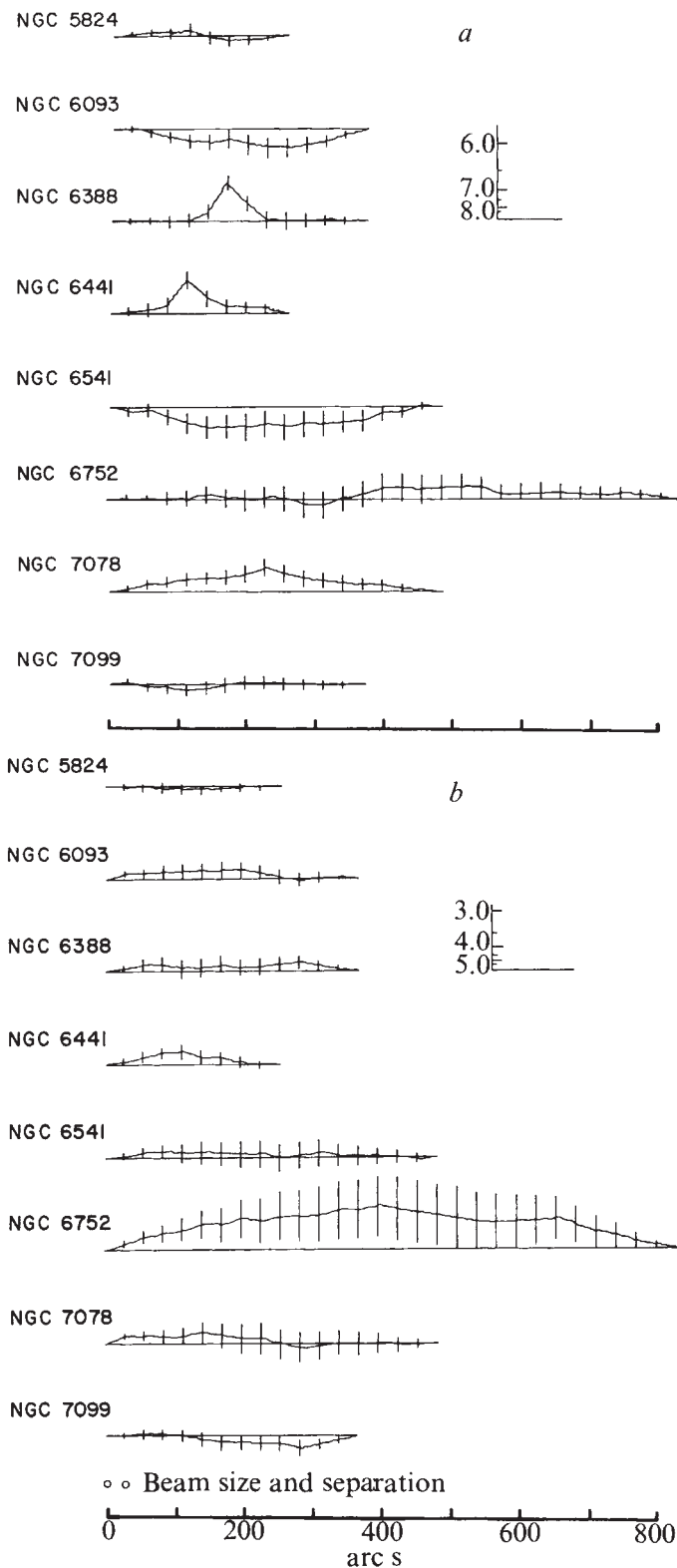


Fig. 1 Average, rectified drift scans of 8 globular clusters at 2.3 (a) and 4.7 μm (b), respectively. Error bars indicate the r.m.s. deviation among individual scans. Each point is treated separately, that is point-to-point coherence has not been invoked. The endpoints of each individual scan have been assumed to be zero; the error bars therefore decrease artificially towards both endpoints. The magnitude scale inserted in the upper right-hand corner of each figure refers to height above the baseline for a uniform brightness distribution across the 10'' aperture.

find any such interstellar material, drift scanning offered certain advantages for a pilot study such as this.

From these observations, shown in detail in Fig. 1a and b, we find that the central regions of the two massive, metal-rich clusters NGC 6388 and 6441 (refs 6 and 7) were detected at 2.3 μm but probably not at 4.7 μm . Taking the errors into consideration, the amplitude of the emission may plausibly be attributed entirely to the giants contained within the sampled volume, provided the luminosity function tabulated by Allen⁸ and the core and tidal radii derived by Illingworth⁶ and Peterson and King⁹ are applicable. Similarly, the failure to detect significant radiation at 4.7 μm is consistent with the 2.3- μm flux being due to radiation characterised by $T_{\text{eff}} > 1,000$ K. As dust would probably be cooler, the observed radiation is more likely of stellar origin. With the possible exception of NGC 7078 (M 15), all other clusters in Fig. 1 and Table 1 (which are those in the upper right-hand portion of Bahcall and Ostriker's³ figure), yield null results within the limits of this study.

Earlier observations at 2.3, 4.7, 10 and 20 μm of NGC 104 (47 Tucanae), 1851, 2808 and 5139 (ω Centauri) failed to show any emission, but the detection limits were considerably less significant than those of the more recent short wavelength observations and they have not been included here. Clearly, important clusters such as NGC 104 (massive and metal rich^{6,10}) and NGC 1851 (a transient X-ray source¹¹) deserve much closer future attention, as do NGC 6388 and 6441.

Finally, should the intriguing observation of a 10- μm emission source in NGC 7078 (M 15) (refs 12 and 13) be confirmed, the present observations may be used to set limits on the (2.3–10) and (4.7–10)- μm colour indices of the emitter.

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Table 1 Observational data

NGC	Scans	Steps per scan	2.3 μm		Scans	Steps per scan	4.7 μm	
			Time per step(s)	Apparent magnitude			Time per step(s)	Apparent magnitude
5824	40	10	91	> 8.8	44	10	100	> 5.5
6093	46	14	95	> 9.0	47	14	97	> 5.1
6388	38	14	102	> 6.7	38	14	102	> 5.1
6441	42	10	100	> 6.9	43	10	102	> 4.7
6541	32	18	84	> 9	33	18	87	> 5.0
6752	25	30	95	> 7.9	25	30	95	> 3.4
7078	51	18	99	> 7.2	49	18	95	> 4.8
7099	49	14	101	> 9	54	14	112	> 5.5

Palaeomagnetic evidence from DSDP cores of northward drift of India

EVIDENCE of latitudinal changes associated with the northward movement of India during the Cainozoic have been described¹ from remanence inclination measurements made on sediments and basalts recovered at DSDP sites in the Arabian Sea (Fig. 1). When combined with palaeomagnetic data from continental India²⁻⁴ the results showed an initial northward movement at a mean rate of 26 cm yr⁻¹ from the Cretaceous to at least Middle Eocene times and a subsequent slower mean rate of 16 cm yr⁻¹ being resumed in the Miocene (Fig. 2a). The original analysis implied a possible pause in motion between the two phases during the Oligocene, for which period no data were available.

Further palaeomagnetic measurements have been completed by us following the recovery during Leg 24 (ref. 5) of Oligocene sediments and basalts at Deep Sea Drilling Project (DSDP) site 238 on the southern edge of the Indian Plate (Fig. 1). Only those samples considered the most stable after demagnetisation are included in the inclination values in Table 1. Palaeolatitudes from site 238 do not seem to support the phase of rapid post-Middle Miocene plate motion (Fig. 2b) but indicate instead that India may have already been much closer to its present latitudinal position by the early Oligocene.

There are several possible reasons for the discrepancy in Oligocene and post-Oligocene palaeolatitudes.

Non-verticality of coring. Inclination measurements at several sites during DSDP Leg 25 (ref. 6) indicate that the holes were never more than 5° off the vertical. Assuming a similar near verticality for the DSDP sites discussed here, errors in palaeolatitudes (probably a maximum of 2-3°) associated with non-vertical holes are, therefore, inadequate to account for the observed differences between Oligocene and post-Oligocene palaeolatitudes. There is no positive indication that DSDP site 238 has been subjected to any appreciable post-depositional tectonic tilting which would affect the observed remanent inclinations. All visible bedding relationships throughout the lower part of the core are horizontal or near horizontal.

Averaged geomagnetic field configuration other than a geocentric axial dipole. All palaeolatitudes considered so far have been calculated from mean absolute inclination values assuming a geocentric axial dipole approximation ($\tan \lambda = \frac{1}{2} \tan I$). Wilson⁷ considers, however, that the geomagnetic field for the Quaternary and Tertiary (as far back as the Miocene) may be better described by an offset dipole, the northward displacement of the dipole during these periods being 191 ± 38 and 306 ± 41 km respectively. All calculated palaeolatitudes younger than the Miocene may therefore be too far south by up to 4° as a result of this offset. Palaeolatitudes have been recalculated from mean absolute inclinations assuming a displaced axial dipole field since the Miocene (Fig. 2c). A rapid though somewhat decreased drift is still apparent for the post-Oligocene sites, requiring a 15° latitudinal change since Middle Miocene times. The offset dipole model then may account for some of the observed disagreement but the revised palaeolatitudes are still broadly inconsistent with the data from DSDP site 238.

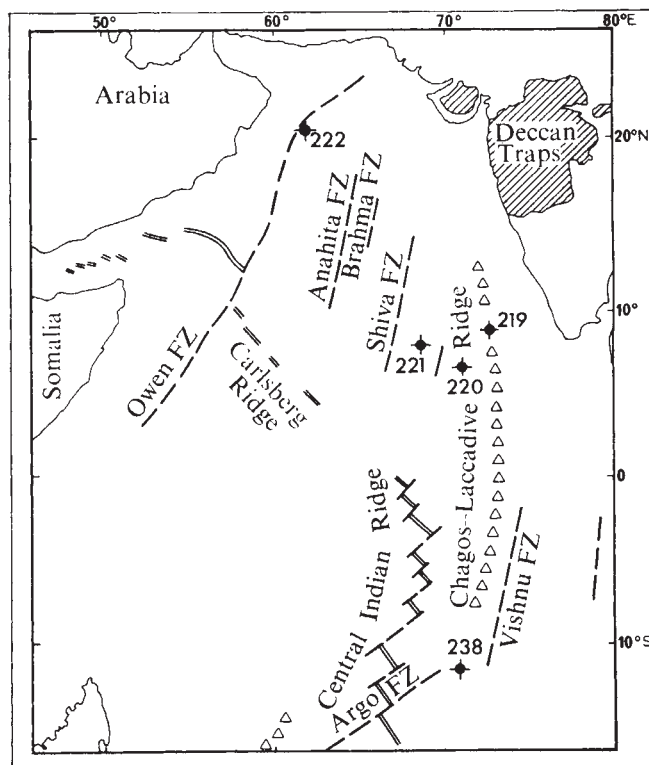


Fig. 1 Location of DSDP site 238 and other DSDP sites in the Arabian Sea which lie on the Indian Plate. FZ, Fracture zone.

Primary remanence deviations. Opdyke and Henry⁸ showed that remanence inclination errors⁹ were not present in some 52 deep-sea sediment cores. The sediments of those cores are, however, predominantly slowly accumulated pelagic and hemipelagic sediments and biogenic oozes for which average sedimentation rates of 10 m Myr⁻¹ are typical¹⁰. For current deposited deep-sea sediments, containing a predominantly terrigenous component and characterised by much faster sedimentation rates, significant inclination errors have been recorded^{11,12}. The mode of acquisition of depositional remanent magnetisation (DRM) is not yet fully understood but a shallowing of inclination might be expected due to gravitational couples (a true inclination error as defined by King⁹), or hydrodynamic tangential shearing of remanence carriers on deposition (rotation effect¹³), to post-depositional compaction, or to any combination of these. Further detailed analysis of the mean inclinations exhibited by terrigenous sediments is required to confirm the presence of such primary remanence deviations. If the possibility of significant inclination errors being associated with terrigenous sediments is acknowledged then the effect of such errors on the DSDP sediments reported here can be considered.

Sediments from DSDP sites 238 (nannochalk oozes, sedimentation rate = 12.7 m Myr⁻¹)⁵, 220 (laminated chalks, sedimentation rate = 19 m Myr⁻¹)¹⁴, and the Pliocene-Miocene sequence from DSDP site 221 (pelagic brown clay,

Table 1 Palaeomagnetic results for DSDP site 238

Site	Material	No. of samples	Age	Mean absolute inclination	Observed palaeolatitude	Reduced palaeolatitude*
238	Sediments	22	Early Miocene-late Oligocene 19-30 Myr	$30.5 \pm 3.2^\circ$	$16.4 \pm 2.0^\circ\text{S}$	$3.7 \pm 2.0^\circ\text{N}$
	Basalts	48	Early Oligocene 34-38 Myr	$28.2 \pm 1.0^\circ$	$15.0 \pm 0.6^\circ\text{S}$	$5.1 \pm 0.6^\circ\text{N}$

All errors are s.e.

*Palaeolatitudes reduced to the common latitude of DSDP site 219 according to the method of Whitmarsh *et al.*¹.

Table 2 Inclination error corrections and corresponding reduced palaeolatitudes

Site	Age	Mean inclination	Inclination error	Corrected palaeolatitude	Reduced corrected palaeolatitude
219	Upper Palaeocene	$29.1 \pm 5.6^\circ$	20.1°	$30.1 \pm 5.7^\circ\text{S}$	$30.1 \pm 5.7^\circ\text{S}$
221	Pleistocene	$15.2 \pm 5.9^\circ$	14.3°	$15.8 \pm 6.1^\circ\text{N}$	$16.8 \pm 6.1^\circ\text{N}$
222	Lower Pliocene	$22.1 \pm 4.9^\circ$	18.1°	$22.9 \pm 5.1^\circ\text{N}$	$11.8 \pm 5.1^\circ\text{N}$
Lower Siwaliks	Upper Miocene	$18.8 \pm 1.7^\circ$	16.5°	$19.5 \pm 1.8^\circ\text{N}$	$8.4 \pm 1.8^\circ\text{N}$
	Middle Miocene	$28.1 \pm 2.1^\circ$	19.9°	$29.1 \pm 2.1^\circ\text{N}$	$5.3 \pm 2.1^\circ\text{N}$

sedimentation rate = 2 m Myr^{-1})¹⁴ are of similar lithology and have sedimentation rates comparable with those sediments described above which are known to record accurately the ambient field inclination value at the time of sediment formation: mean inclinations from these sites thus provide a reliable estimate of site palaeolatitude during the corresponding stratigraphic intervals. In contrast, sediments sampled for palaeomagnetic study at DSDP sites 222 (grey detrital silty clays, sedimentation rate = $135\text{--}600 \text{ m Myr}^{-1}$)¹⁴, 219 (sandstones and siltstones, sedimentation rate $> 70 \text{ m Myr}^{-1}$)¹⁴ and the late Pliocene–late Pleistocene turbidite sequence from DSDP site 221 (silt, sedimentation rate = 170 m Myr^{-1})¹⁴ are all rapidly deposited terrigenous sediments with which significant inclination errors may be associated. An initial estimate of the magnitude of such inclination errors may be obtained by a consideration of the true inclination error predicted by the rolling spheres model¹⁵. The mean observed inclination (I_0) is related to mean geomagnetic field inclination (I_f) by the expression¹⁶:

$$\tan I_0 = 0.48 \tan I_f$$

from which the computed value of I_f has been used to calculate a revised palaeolatitude, assuming again a geocentric axial dipole model. Inclination error corrections, where applicable, together with the revised palaeolatitudes are given in Table 2. The directions of magnetisation of the Lower Siwalik detrital red shales are considered to have been acquired during deposition²: accepting a DRM origin for these rapidly deposited¹⁷ continental sediments an inclination error correction has been applied as above. All corrected site palaeolatitudes again reduced to the common latitude of DSDP site 219 are shown plotted in Fig. 2d. Allowance for inclination error effects may be seen to improve markedly the internal agreement of the palaeolatitude data from these DSDP cores. A possible overestimation of inclination errors in natural sediments by the rolling spheres model may account for the more northerly placing of the palaeolatitudes of DSDP sites 221 and 222 than is expected from their present site latitudes.

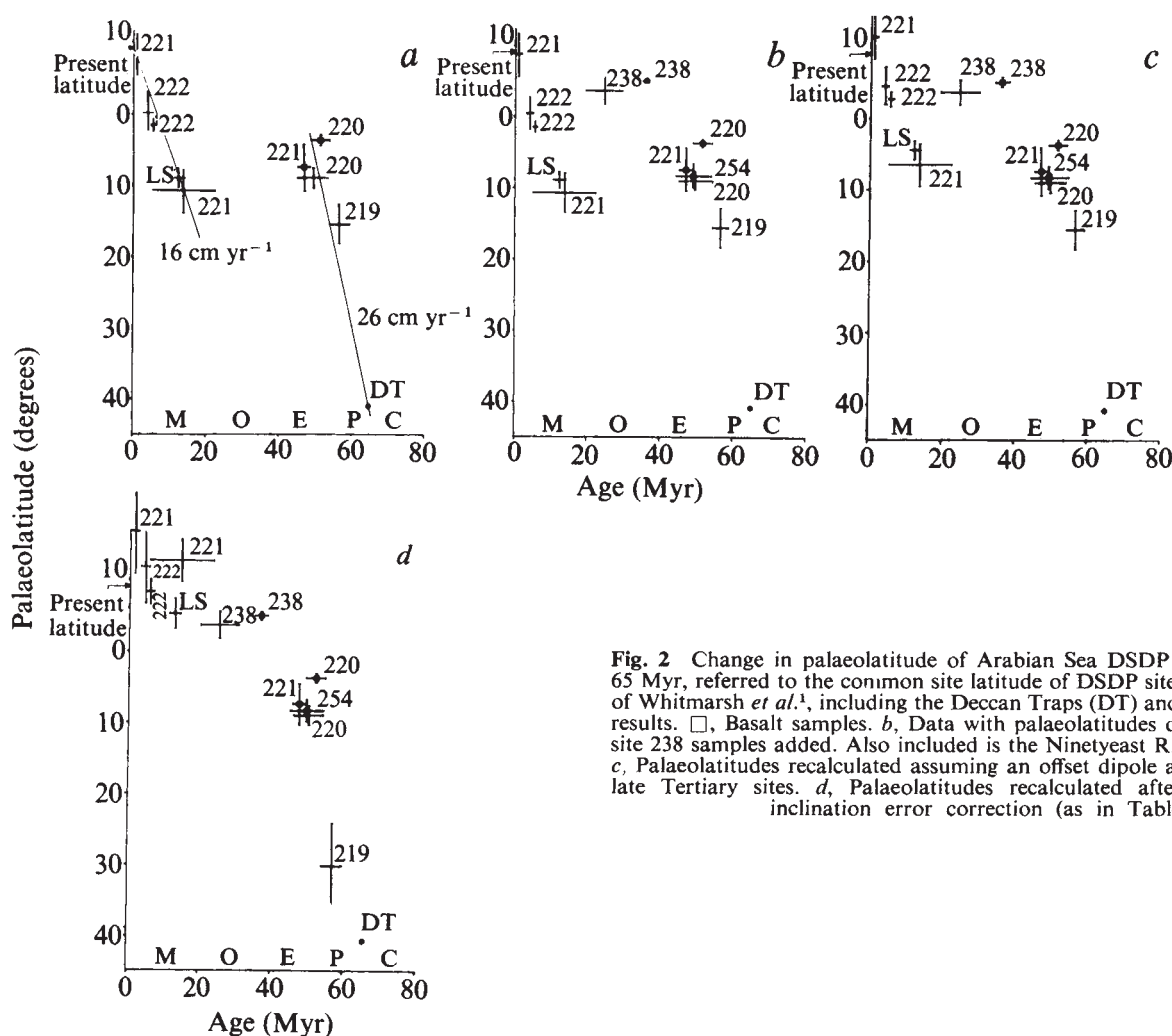


Fig. 2 Change in palaeolatitude of Arabian Sea DSDP sites during the past 65 Myr, referred to the common site latitude of DSDP site 219. *a*, Original data of Whitmarsh *et al.*¹, including the Deccan Traps (DT) and Lower Siwaliks (LS) results. □, Basalt samples. *b*, Data with palaeolatitudes determined for DSDP site 238 samples added. Also included is the Ninetyeast Ridge site 254 (ref. 20). *c*, Palaeolatitudes recalculated assuming an offset dipole approximation for the late Tertiary sites. *d*, Palaeolatitudes recalculated after application of an inclination error correction (as in Table 2).

The revised northward drift history of the Indian plate during the late Tertiary indicated by the DSDP cores described here is broadly in agreement with the Australian palaeomagnetic data^{18,19}. It provides an opportunity to qualify further the time at which the early Tertiary phase of rapid spreading ceased and the nature of the motion since that time. From the palaeomagnetic standpoint this assessment of palaeolatitudes has indicated that inclination errors may yet be of importance in certain clastic sedimentary formations.

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Seismic evidence for local undulation of olivine-spinel phase boundary

A DETAILED P-wave velocity distribution has been determined (S.D.-S. and R.A.W., unpublished) for the upper mantle in western Canada. The analysis was based on differential travel-time and relative-amplitude observations of multiple arrivals for seismograms between 14 and 40°. Three velocity models were determined for three different regions. All of these models are characterised by discontinuities at 410 and 650 km. The reflection from the 410-km discontinuity is best observed between 14 and 18°. While analysing seismograms between 14 and 18°, a group of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation of 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.

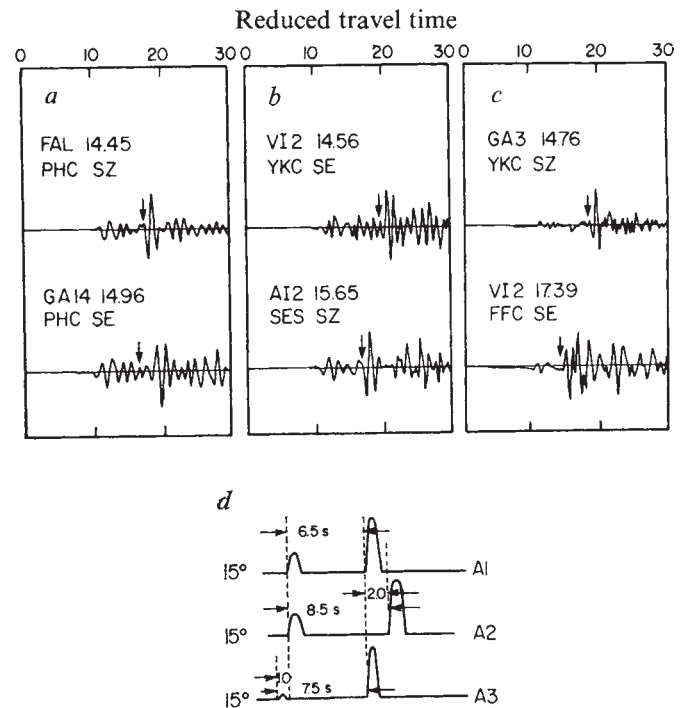


Fig. 1 Some typical seismograms of types A1 (a), A2 (b) and A3 (c) in group A (14–18°). d, Schematic representation at 15°. FAL, Faultless; VI, Vancouver Island; GA, Gulf of Alaska; AI, Alaskan Islands.

Figure 1 shows some typical seismograms around 15°, designated as group A type and subdivided into, A1, A2 and A3. The abbreviated event name and the epicentre distance are written at the top, the station name and the component of motion are written below. The name of the event roughly corresponds to its geographical location. The first arrival is always aligned at the 10.0 mark. Figure 2 shows the spatial locations of all observations. Each arrow represents the source station midpoint and the direction of the arrow indicates the direction in which the ray is travelling at its turning point. This midpoint represents the surface projection of the turning point of the ray at depth.

The amplitude ratio of second to first arrival is about 3 : 1 in A1 (Fig. 1a) and A2 (Fig. 1b) type records, in contrast to about 10 : 1 in A3 (Fig. 1c) type records. This difference in relative amplitude behaviour between A1 and A3 records was responsible for the two velocity models WCA and WCB. In model WCB, the velocity at the base of the low velocity zone is 8.4 km s⁻¹ compared with 8.17 km s⁻¹ in model WCA. This causes the first arrival to arrive about 1 s early between 14 and 18° with respect to that in WCA. As can be seen from Fig. 2, model WCA is valid for the ocean and the region near the ocean-continent boundary, whereas model WCB is valid for regions east of the Rocky Mountain trench.

The A2 type seismograms, which are similar to A1 types as far as relative amplitude is concerned, are observed

Table 1 First arrival time data for A1 and A2 seismograms for distances around 15°

Event	Station	Distance (°)	Travel time (s)	Reduced travel time at 15° (s)	Mean (s)	Seismogram type
VI3	YKC	14.81	210.0	212.40	211.5	A2
VI4	YKC	14.97	210.5	210.90		A2
VI5	YKC	15.05	212.0	211.40		A2
Faultless	PHC	14.45	204.0	210.90	211.7	A1
Handley	PHC	15.52	217.0	210.50		A1
GA14	FSJ	14.60	208.5	213.50		A1
GA14	PHC	14.96	211.6	212.10		A1

VI, Vancouver Island; GA, Gulf of Alaska.

in the region in between WCA and WCB. They are different from A1 types in that the delay between first and second arrival is greater in A2 types for the same epicentral distance. To obtain a clear picture of the delay, it is necessary to reduce all the observations to a single distance, say 15° . In Fig. 2 beside each arrow, the reduced delay times at 15° are plotted. There exist three types of delay between 14 and 18° . The delay is 6.5 s for A1 types, 8.5 s for A2 types and 7.5 s for A3 types. A representation of all three record types at 15° is presented in Fig. 1d. The differences between A1 and A3 records can be explained on the basis of higher absolute velocity at the base of the low velocity zone, causing the first arrival to appear one second earlier. That is not the case, however, with A2 type records. The first arrival data for A1 and A2 type records around 15° are shown in Table 1. Within observational accuracy the first arrival time is 211.0 s at 15° . This means that the second arrival is 2.0 s late in comparison to A1 types. Since the second arrival is a reflection from the 410 -km discontinuity its lateness can be explained by assuming a downbuckling of the 410 -km discontinuity.

To examine this hypothesis, the 410 -km discontinuity in model WCA was displaced by 30 km and synthetic seismograms were generated by quantised ray theory⁶. The synthetics were generated for a unit impulse and then convolved with a half lobe of a sine wave of frequency 1 Hz. It was observed that in the synthetic seismograms the delay is 8.5 s at 15° and the relative amplitudes compare very well with A2 type seismograms. But this shift in the discontinuity also produces significant changes in the wide angle reflections. When the discontinuity is at 410 km, the wide angle reflections from it appear as second arrivals between 22.5 and 23.5° with a delay of 5.0 – 6.0 s. When the discontinuity is at 440 km, however, these wide angle reflections continue up to 25° . At 25° there should be three arrivals, the third arrival being the wide angle reflection, arriving 10.0 s later than the first arrival. Unfortunately, as present source–station locations stand, it is very difficult to obtain observations at 25° such that the source–station midpoint will be in the same location as the A2 type seismograms.

So even though the hypothesis of local undulation does satisfy A2 type seismograms, it cannot be confirmed in the absence of observations at 25° around the precise location of this anomalous behaviour in A2 type seismograms.

The discontinuity at 410 km is believed to be due to a phase change from olivine to spinel¹. From the point of view of convection current hypotheses in the mantle³, this phase boundary will be lower where material is rising up (mid-oceanic ridges) and will be higher where material is going down (subduction zones). Along the western part of the North American continent, a remnant of a ridge system (East Pacific Rise) does exist. If the downbuckling of the 410 -km discontinuity is due to a ridge system, then it should be observed elsewhere beneath western North America. This is not the case. The spatial location of the downbuckling is localised in central British Columbia, seemingly invalidating this explanation.

In Fig. 2, the hatched area represents the region of Cainozoic and Mesozoic volcanic activity along with some sedimentary formations. The location of downbuckling is very close to this large scale volcanic activity. The volcanics are of the alkali–olivine type⁵, where olivine is generally more rich in iron than in magnesium⁴. If by localised partial melting at a depth of 410 km the olivine is enriched in magnesium with respect to the surrounding region, then it will require a higher pressure to achieve the phase transformation to spinel. Thus the discontinuity will show a local depression. This partial melting of the olivine will then generate the iron-rich alkali–olivine basalts observed on the surface. The obvious flaw in this argu-

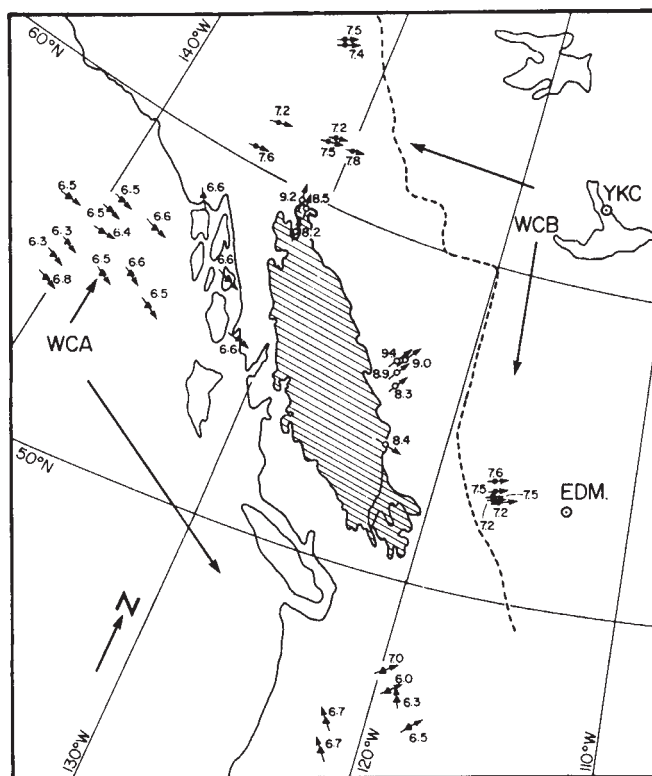


Fig. 2 The spatial locations of A1, A2 and A3 type seismograms. Hatched area shows the location of Cainozoic and Mesozoic volcanic activity. ▲, A1 type; ●, A3 type; ○, A2 type. The numbers beside each arrow indicate reduced delay times at 15° .

ment is the assumption that alkali–olivine basalts were generated by partial melting at a depth of 410 km. So far the evidence for the maximum depth of partial melting as determined by a study of cognate xenoliths gives a depth of about 200 km (ref. 1).

We thank Dr Henry Halls at the University of Toronto for directing our attention towards the presence of volcanics with the location of undulation of 410 -km discontinuity.

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Sedimentary provinces of the Saronic Gulf system

A PRELIMINARY survey of present-day sediment distribution within the Saronic Gulf system, off the coast of Greece, has been made in support of an ongoing study being conducted by the Institute of Oceanographic and Fishing Research (IOKAE). Here we present the first sedimentary province

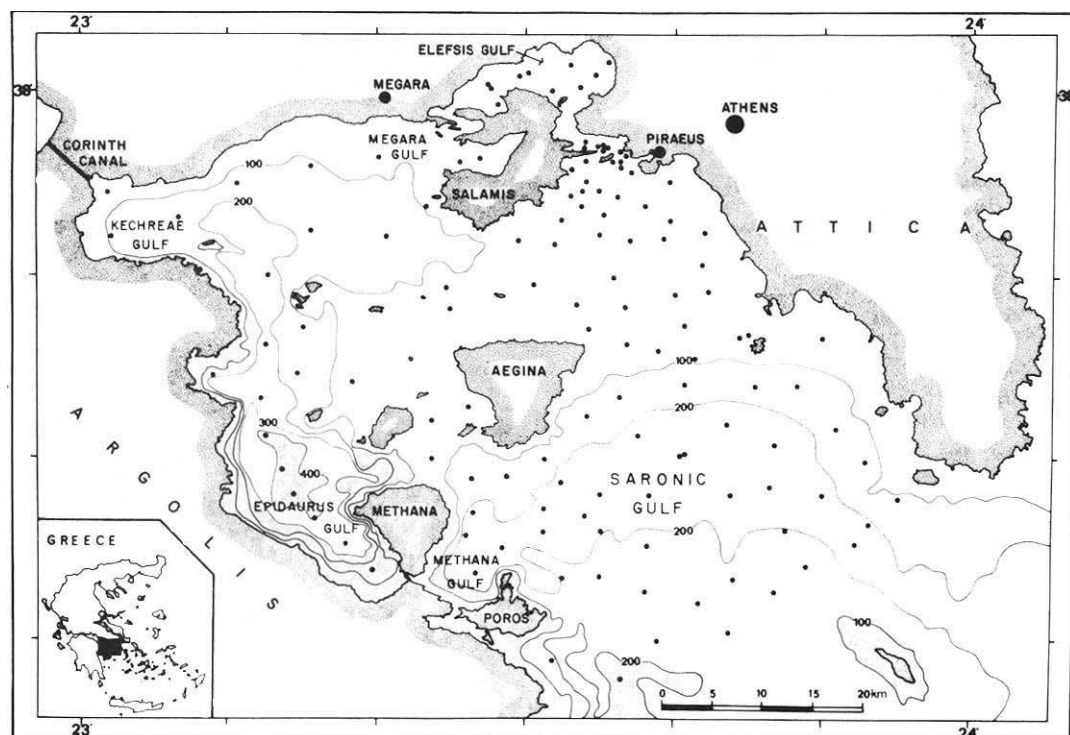


Fig. 1 Location, bathymetry and sample site map.

map of the gulf system, which expands current knowledge of the region and serves as a basis for further studies.

The only previous work of this nature done in the several gulfs comprising the Saronic system was contained in a biologic investigation by Vamvakas¹. He analysed 14 sediment samples from a limited area to determine the weight percentage of the coarse and fine fractions. Our observations confirm the results of his investigation concerning sediment characteristics and it is hoped that future research will delineate further the geological parameters and processes of the Saronic Gulf system.

The Saronic Gulf system as defined here incorporates the Saronic Gulf proper, as well as the Methana, Epidaurus, Kechreæ, Megara, and Elefsis gulfs (Fig. 1). It is bounded

on the west by the Argolis Peninsula of the Peloponnesus, on the north and east by Attica, and opens to the south-east into the Myrtoon Sea. The seaward limit of the survey area is taken as a line from the south-eastern tip of Argolis to the southern extremity of Attica. The system has an area of 2,900 km² and, as compared with calculations by Hatzikakidis², comprises of the order of 10% of the continental shelf of Greece.

The depth of the eastern sector of the region ranges from <100 m in the Piræus–Athens area, to between 100 and 200 m between Aegina and Attica, and is >200 m in a trough that extends from off the tip of Attica into the Methana Gulf. To the south, the depth shoals to less than 200 m. Elefsis Gulf is relatively shallow, less than 40 m

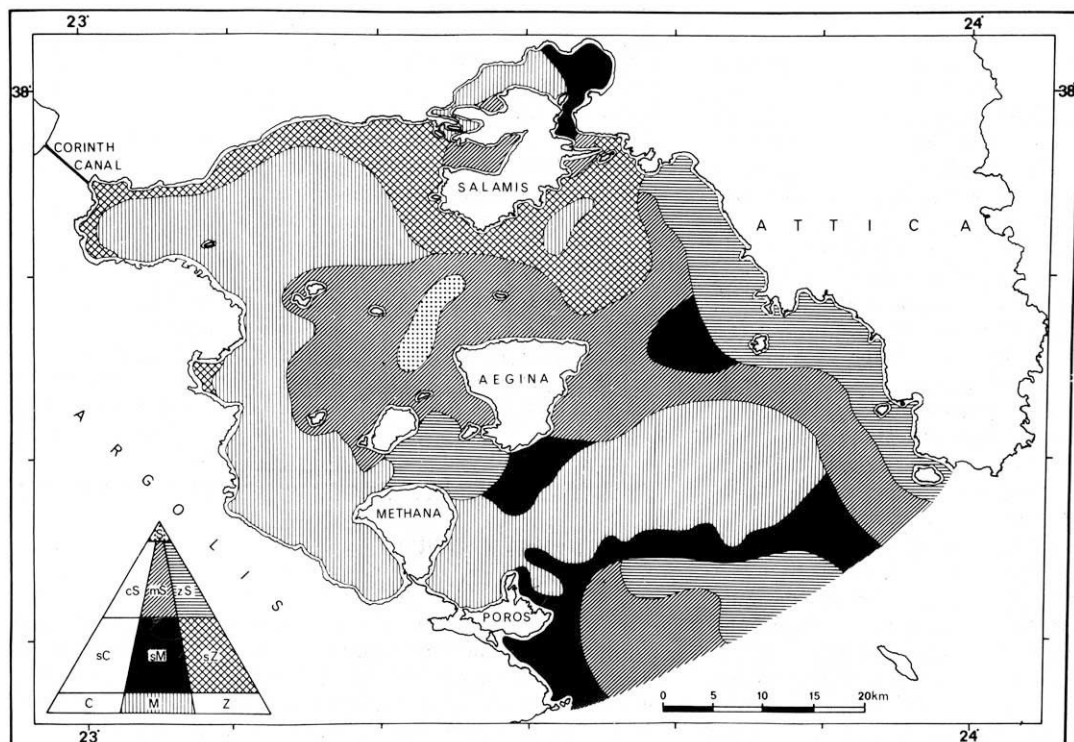


Fig. 2 Sediment distribution within the Saronic Gulf system. S, sandy; cS, clayey sand; mS, muddy sand; zS, silty sand; sC, sandy clay; sM, sandy mud; sZ, sandy silt; C, clay; M, mud; Z, silt.

throughout. West of Salamis the Megara and Kechreai Gulfs exceed 100 m, and connect in the west with a north-south deep that reaches a depth of > 400 m in the Epidauros Gulf between Methana and Argolis. The Epidauros Gulf deep and the Methana Gulf-South Saronic Gulf trough seem to bear a close correlation to pre-Pleistocene and Pleistocene normal faults, respectively, which transect the region. Further characteristics of the Saronic Gulf system have been described previously^{3,4}.

Source water to the Saronic Gulf is provided by a western boundary current in the Aegean Sea which flows past Attica into the gulf, and the annual circulation within the Saronic Gulf system has been investigated⁵. Wind generated waves are of limited fetch in all but the south-easterly direction, although seiches are occasionally generated by pressure disturbances passing over the area. Winds and barometric pressure changes contribute to a very slight Saronic Gulf tide with a range of up to 50–60 cm between the extreme high and low water marks. There are no major rivers draining into the basin and run-off from rivers and streams is insignificant except during periods of heavy, but short lived, rainfall which occur between late autumn and early spring.

The sampling programme was carried out during cruises aboard the Greek Hydrographic Service vessel Anemos and the Greek Navy minesweepers Antiopi, Niovi, and Phaedra. Altogether, 131 bottom-samples were taken (Fig. 1) using Petersen, van Veen, and clamshell snapper grab samplers.

The colour of each bulk sample was determined by comparison with the standard rock colour chart⁶. The portion of each sample subjected to particle size distribution analysis was first thoroughly mixed in a sodium hexametaphosphate (Calgon) solution (5.5 g l^{-1}), to achieve maximum dispersion. The sediment was then wet-sieved to collect the coarse, sand, fraction between 0.062 and 2 mm. The sand fraction was subsequently examined with the aid of a binocular microscope to ascertain its textural and compositional characteristics. For the purposes of this investigation an arbitrary classification was established whereby the sand fraction was called terrigenous if mineral and/or rock fragments comprised 40% or more, by estimated weight, of the fraction. No attempt was made to identify the organisms which constituted the organic component of the sand fraction (Vamvakas¹ has reported on this for the sector covered by his studies, and our 131 sand fraction samples are now undergoing species identification). Pipette analysis of the fine fraction was carried out, using 20-ml aliquots of the 4ϕ and 8ϕ classes at the appropriate times and depths, to determine the clay and silt content of the sample on a weight per cent basis. Based on these analyses of the sand-silt-clay ratio, each sample was then classified according to the system of Folk⁶.

It is assumed that basic factors affecting sediment distribution in this region are as follows. First, coarse terrigenous sediments, where present, are transported by wave action in coastal and shoal areas. The fine fractions settle in deep quiet waters or areas of low wave energy. Second, planktonic productivity increases toward the nutrient-rich waters in the north⁷. Benthic productivity, the main source of the biogenous coarse fraction, decreases with depth; a notable change taking place at the 200-m bathymetric contour¹. Third, yellow sediments predominate throughout the system. Where present, olive-coloured sediments denote reducing conditions caused either by poor circulation at depths or by wastes rich in organic matter, or contain ferromagnesian detritus. Finally the introduction of waste material into the system may inhibit benthic community growth and/or add fine particulate matter to the water column.

Figure 2 shows the sediment distribution within the Saronic Gulf system based on the locations of our 131 samples.

The investigation outlined here was conducted under the aegis of the Institute of Oceanographic and Fishing Research in Athens, with the cooperation of the Greek Navy and the Greek Hydrographic Service. M.L.S. was also supported by the Fulbright-Hays programme. We thank George Marinos, Ilias Mariolakas, Fotini Dousgou, and Joy Dabney for assistance.

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Stress on the bottom of an estuary

MANY estuaries are sufficiently deep relative to their maximum fetch that even in severe storms the bottom remains undisturbed by wind-generated waves. Yet observations of sediment transport and of changes in benthic animal populations indicate that agitation of the bottom of deep estuaries is not infrequent (refs 1–3, and D. C. Rhoads, unpublished). We report here an analysis of current meter records which shows that such bottom disturbances can arise because wind stress at the surface causes increased turbulence deep in the water column and, consequently, a more frequent occurrence of unusually high velocities near the bottom.

The records analysed were obtained at two locations along the central axis of Long Island Sound (LIS), an estuary 150 km long, 32 km wide at the widest point and with a mean depth of 17 m. The dominant water movement in LIS is the resonant co-oscillating tide⁴; tidal stream speeds reach 30 cm s^{-1} at the locations studied. Currents were recorded with two types of meters: type I meters recorded instantaneous measurements of water velocity at time intervals which may be set from 1 to 30 min. Type II meters recorded the average speed and direction over successive 20-min intervals. All the meters were operated on taut moorings and were set 2 m above the bottom. The recorded velocities have been resolved into E–W and N–S components, approximately parallel and perpendicular to the axis of the estuary. Each time series of velocities is decomposed into a constant term, a group of periodic terms, and a fluctuating term. The periodic components have been removed from the velocity records by a least-squares regression of 18 sinusoids of tidal periods. The constant term is defined as the mean velocity over 3 or 12 h, depending on the time period chosen for calculating the variance or standard deviation. The residue remaining after removal of the mean and periodic parts is described as the fluctuating component, u' .

Wind data have been obtained from the US National Weather Service station at Stratford Point ($41^{\circ}10'N$, $73^{\circ}08'W$), an exposed location on LIS. The data consist of 3-min averages of speed and direction, recorded every 3 h.

Figure 1 is a polar histogram of u' calculated from a record made by a type I current meter at Station A ($41^{\circ}09'N$, $72^{\circ}48'W$,

depth=23 m) sampling the flow every 5 min for 4 d. The fluctuating velocities are isotropic. The square of the wind speed, proportional to the wind stress, and the standard deviation, σ_x , of u_x' over 3-h intervals are compared in Fig. 2a. The wind was from the north throughout the period of observation. Increases in wind stress result in increases in σ_x but the peaks of σ_x lag those of the wind stress by about 5 h. The time series of the E-W and N-S components of u' are quantitatively similar, as expected from the results in Fig. 1.

Two additional sets of data were obtained by sampling the current less frequently over a period of nearly one month at station B (41°08'N, 72°53'W, depth = 23 m). A type I current meter sampling every 30 min, and a type II meter were used. Wind stress data and σ calculated for 12-h periods are shown

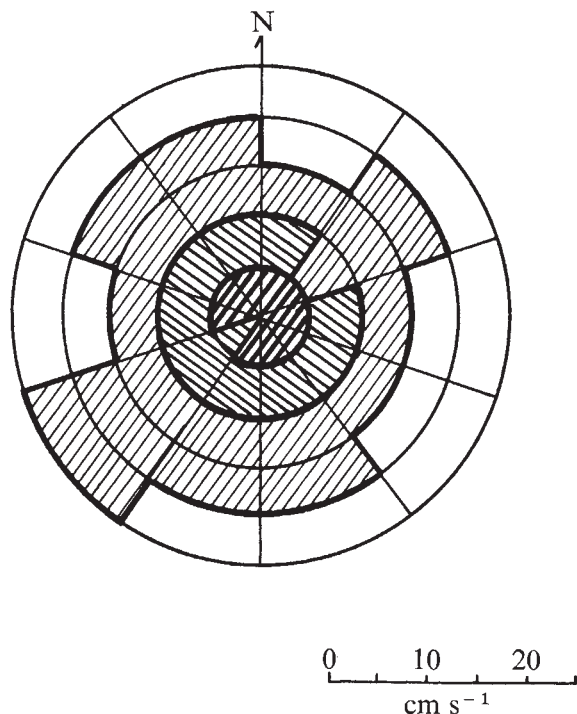


Fig. 1 Histogram of 951 observations of the fluctuating velocity component, u' , at station A. Darkest shading, probability of an observed velocity being in a box is $5\% < P < 7.5\%$; intermediate shading, $2.5\% < P < 5\%$; lightest shading, $0 < P < 2.5\%$. There is apparently no dependence of u' on direction.

in Fig. 2b and c. During calm weather σ drops to the range $1.5\text{--}2\text{ cm s}^{-1}$ identified as characteristic of the turbulence in the undisturbed tidal stream. Small increases in wind stress do not have much effect on σ but winter storms raise it by as much as a factor of 4 above the level associated with the tidal stream alone. The increase appears to be slightly smaller and the background somewhat higher for the type II meter, a consequence of the 20-min averaging performed in the meter itself. The magnitude of σ in the two data series in Fig. 2b and c is insensitive to wind direction. No correlation between u' and the phase of the tide is evident in the records.

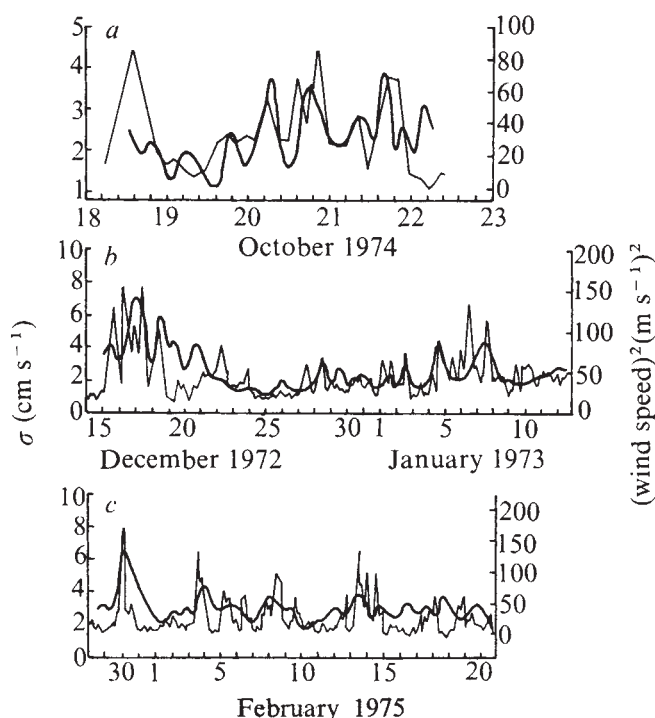
That the observed increase in u' does not result from disturbance of the meters by orbital motion of waves on the surface is shown by two lines of evidence: a water pressure recorder (wave gauge) was operated during the period February 19–March 27, 1975 on a reef at 41°00'N, 73°25'W, in water 14 m deep. Records of pressure fluctuations caused by waves on the surface were obtained when the wave period exceeded 4 s. (The recorder is insensitive to waves of shorter periods because of the large hydraulic attenuation.) There is a close correlation between the amplitudes of the pressure fluctuations and the factor $v^2/f/f_m$ where v is the wind speed, f is the fetch for the

wind direction and f_m is the minimum possible fetch at the site. Throughout LIS large values of f/f_m occur only in the ENE direction. Pressure fluctuations at the recorder are detected only for easterly winds. The fetch in all other directions is too short to allow waves of long period to develop, even for very strong winds. All of the current meters operated in water deeper than that in which the wave recorder was placed so that, if disturbed by waves, the disturbance would occur only for easterly winds. In fact, σ_x and σ_y depend only on the wind speed; they are independent of wind direction. Examination of the magnitude of u' during the major storm of December 14–17, 1972 illustrates that point: strong winds from the ENE, which produced a storm surge with an amplitude of 1.2 m on December 15 were followed by WNW winds which produced a negative surge of 1 m. The magnitude of σ is significantly greater when the WNW winds are prevailing, at times when waves with long periods are absent.

A second line of evidence comes from the observation that the magnitude of u' is isotropic, as shown in Fig. 1, in the record obtained at location A. During the entire period covered by that record the wind direction was constant (from the north). Any direct contribution to u' from wave action should be greatest in the direction of wave advance, which is not observed.

The current meter records show that during periods of high wind stress the frequency of occurrence of high velocities near the bottom is increased. For example, $\sigma = 3.4\text{ cm s}^{-1}$ for the second 8.3 d of the record in Fig. 2b and the probability of occurrence of $|u'| > 10\text{ cm s}^{-1}$ is found from the distribution curve in Fig. 3 to be less than 1%. During the stormy period, the first 8.3 d of the record, $\sigma = 6.7\text{ cm s}^{-1}$ and the probability of encountering $|u'| > 10\text{ cm s}^{-1}$ increases to 12%. In an estuary the average kinetic energy resulting from storm-generated turbulence may be small compared to that generated by the tide, but the more frequent occurrence during storms of speeds greater than the maximum speed of the tidal stream, V , will have a large influence on processes characterised by a critical speed $v_c > V$ (processes such as the transport of the coarser fractions of the sediment). Very large speeds, such as

Fig. 2 Time series of σ (heavy lines) and the square of the wind speed (light lines). a, σ_x for a type I meter at station A with the wind speed advanced to lead the current by 5 h; b, σ_x for a type I meter at station B; c, σ_y for a type II meter at station B.



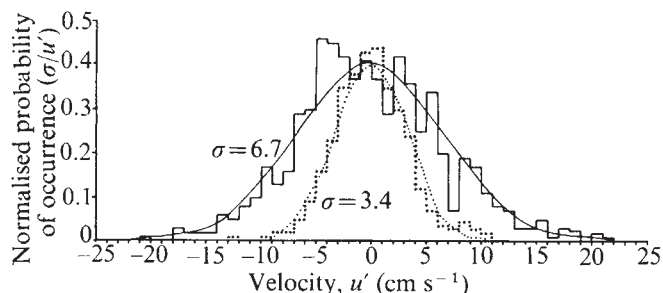


Fig. 3 Distributions of E-W fluctuating velocity components from the combined records of two current meters operating simultaneously 1.5 km apart at station B. Solid histogram data from an 8.3-d stormy period beginning on December 14, 1973; heavy dotted histogram, data for the subsequent, calmer 8.3 d; the solid and dotted curves show the normal distributions with the same mean and variance for each period. Each histogram contains 800 observations.

2V or 3V, are also much more likely to be encountered in storms, with a consequent disruption of benthic animal communities, even though the latter may be well below the depth directly affected by waves.

The data used here were obtained in studies for the New England Division, US Army Corps of Engineers and the United Illuminating Company of New Haven, Connecticut.

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First marine Triassic fauna from the Antarctic Peninsula

THE fauna reported here was obtained from the Legoupil Formation in the north-western part of the Antarctic Peninsula (Fig. 1), which has been assigned a Cretaceous age on the basis of radiometric data^{1,2}. Among the first collection of poorly preserved fossils from this formation were two bivalves tentatively referred to the Cretaceous genus *Platopis*, an identification which substantiated the radiometric evidence. The study of a more recent collection, by members of the British Antarctic Survey, shows, however, that the bivalves have strong Triassic affinities and, since there is no reason to suspect that they are derived, indicates that the Legoupil Formation is of the same age.

It is also noteworthy that the sandstones and greywackes of the Legoupil Formation are lithologically and structurally unlike other known Cretaceous sequences in western Antarctica, more closely resembling rocks of the Trinity Peninsula 'Series'³⁻⁵ of possible late Palaeozoic age⁶⁻⁷. Both formations crop out in close proximity in the area discussed here, and the two have been confused in the field when the same outcrops have been assigned to both formations by different investigators^{2,3}. That reflects the similarity of the two rock groups and casts doubt on whether they are really distinct.

As yet, marine invertebrate fossils are unknown from the Trinity Peninsula 'Series' proper, and those from the Legoupil Formation are only known from a belt 100 m wide on the Duroch Islands (Fig. 1). The fauna is dominated by bivalves which occur mostly as moulds and are usually concentrated in

thin beds or decalcified coquinas. In spite of their unpromising state of preservation, several specimens still show sufficiently fine detail in the hinge region to allow reasonably confident identifications of following: *Myalinella* (?) sp., *Bakevelloides* aff. *hekiensis* (Kobayashi and Ichikawa), *Hoernesia* (?) sp., and *Neoschizodus* sp. nov.

Other fossils include a possible fragment of an inarticulate brachiopod, a gastropod, a serpulid, and some possible arthropod tracks.

Stratigraphically, the two most important species are *Bakevelloides* aff. *hekiensis* and *Neoschizodus* sp. nov. (Fig. 2). The best specimen of *Bakevelloides* (Fig. 2a) shows the ligament pits and pseudotaxodont dentition found in the type material⁸, but the dentition is better developed than in *B. hekiensis* which it otherwise resembles closely. Most species of *Bakevelloides* described seem to occur in the Upper Triassic of Asia. *Neoschizodus* sp. nov. (Fig. 2b) is the commonest element in the fauna. It shows typical myophoriid dentition and has a prominent myophorous buttress. It is closely related to the widely distributed *N. laevigatus* (Ziethen) of Lower to Middle Triassic age, but differs in having a radial riblet on the posterior area, and in possessing striations on some teeth. No specimens that

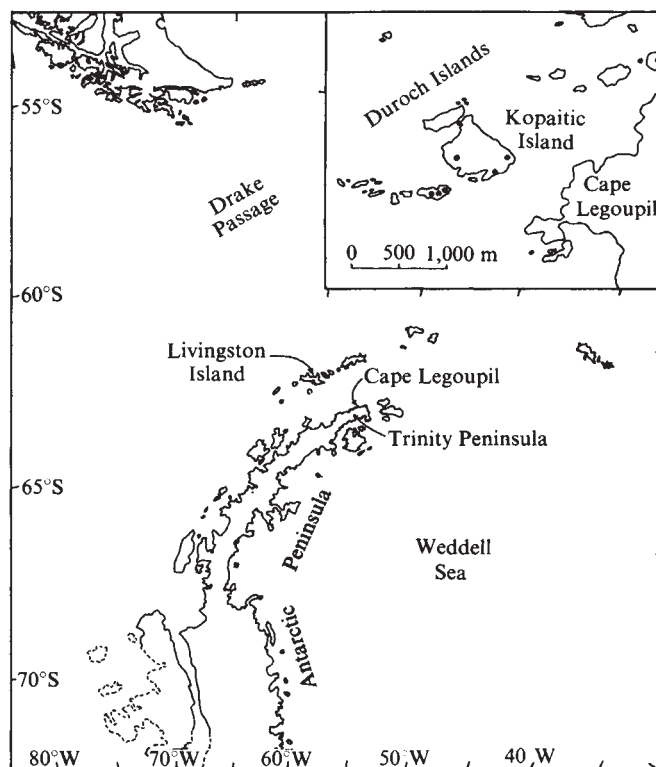


Fig. 1 Map of the Antarctic Peninsula showing the location of Cape Legoupil; inset, fossiliferous localities on the off-lying Duroch Islands (solid circles).

could be referred to *Platopis* (?) sp. were identified among the collection. The only illustrated example of that species^{1,2} compares closely with the specimens here referred to *Neoschizodus*, and it is suggested that earlier ascriptions to *Platopis* resulted from a misidentification.

This fauna provides the first evidence of marine Triassic rocks in the Antarctic Peninsula. The only previous fossil evidence for Triassic deposits in western Antarctica came from the discovery of poorly preserved plant fossils on Livingston Island¹⁰ but these were not collected *in situ*. Because of the close lithological and structural similarities between the Legoupil Formation and the Trinity Peninsula 'Series', it now seems possible that the upper age limit of the latter rock group could be younger than envisaged previously. Some tenuous supporting evidence has been cited from Livingston Island,

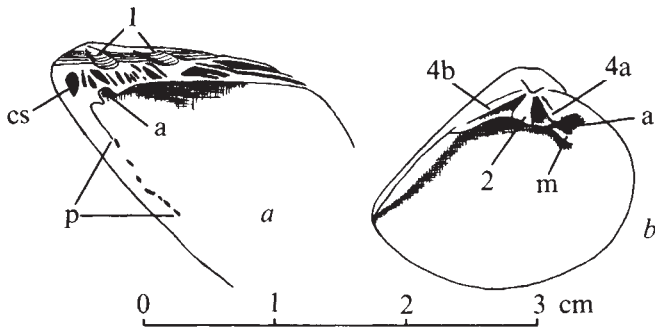


Fig. 2 Fossils from the Legoupil Formation: *a*, *Bakevellioidea* aff. *hekiensis* (Kobayashi and Ichikawa); *b*, *Neoschizodus* sp. nov.: *a*, anterior adductor muscle scar; *cs*, cardinal socket; *l*, ligament pits; *m*, myophorous buttress; *p*, pits along palial line.

where poorly preserved macroplant remains occur in the Miers Bluff Formation, previously correlated with the Trinity Peninsula 'Series' of the Antarctic Peninsula. That material suggests an age later than Carboniferous, perhaps even Mesozoic¹¹.

A detailed account of the fauna from the Legoupil Formation, and its implications, will be published elsewhere¹².

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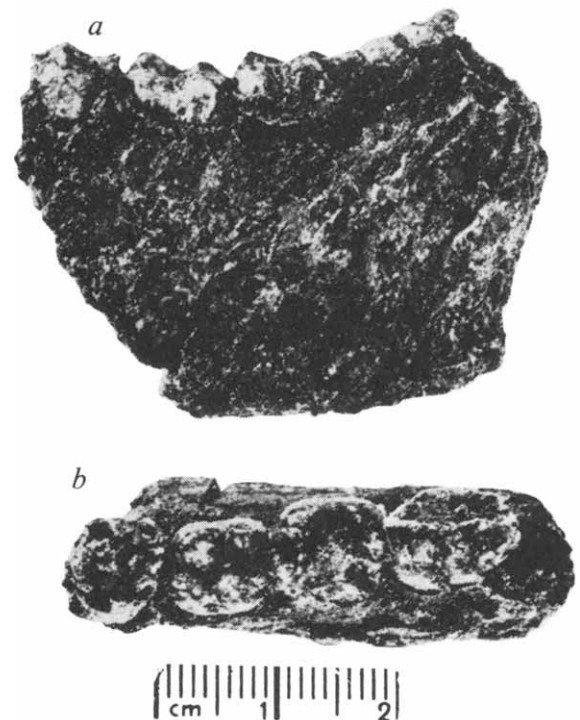
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very rich Lower Pliocene site, Rudabánya in north-eastern Hungary, recently yielded anthropoid material. The fossil-bearing strata belong to the lignitic Pannonian (Lower Pliocene) limnic sediments overlying the karstic depressions and valleys of the Lower-Middle Triassic range of the Rudabánya Mountains impregnated by a metasomatic iron ore body. The Pannonian strata contain, apart from the primate specimens, a very rich vertebrate fauna, a good mollusc fauna and a very rich flora (details of which will all be published elsewhere). The locality was rediscovered as an outstanding site of fossil hominoids by Mr G. Hernyák, chief geologist of the Iron Ore Works of Rudabánya, who has also collected the most important hominoid remains.

The flora indicates Mediterranean-subtropical climatic condition with traces of zonation ranging from aquatic through wet forest to grassland and higher mountain elements respectively. The basically Lower Pliocene character of the vertebrate fauna is proved by the presence of *Hipparion* in the fossil material. That it is a *Hipparion* fauna of archaic character is confirmed by the persistence of Miocene insectivore elements (*Trimylus*, *Miosorex*, *Dimylechinus*, *Plesiodimylus*, *Galerix*, *Lantanothierium*), the three hominoid forms, and especially the Miocene *Pliopithecus*, cricetids of Mio-Pliocene transitional type (*Cotimus*, *Democricetodon*), varied amphicyonids (*Amphicyon*, *Agnotherium*, *Rudacyon* n.g.), definitely Miocene carnivores (*Semigenetta*, *Sansanosmilus*), or archaic lagomorphs (*Amphilagus*). At the same time, forms typical of younger *Hipparion* faunas are entirely missing, (for example, *Mesopithecus*, *Chalicomys*, *Neocricetodon* "Kowalskia") and especially all kinds of murids, hystricids and leporids, or icitheriines, hyenas, true *Machairodus* forms, large agriotheriids (*Indarctos*, *Agriotherium*, *Agriarctos*), among ungulates primarily suids of 'Microstonyx' type, varied antelopes and all gazelles. All this evidence indicates that the fauna resembles that of Eppelsheim (Eppelsheimian), but certainly antedates it (Bodvaian) and thus should be dated as more or less synchronous with the invasion of *Hipparion* in Europe.

The anthropoid material is from 20 individuals (84 teeth altogether and 18 postcranial fragments) representing three different forms. One is a *Pliopithecus* species of great size

Fig. 1 *Rudapithecus hungaricus* Kretzoi, right mandibular corpus, Rud-1 (type). *a*, Lateral; *b*, occlusal view ($\times 1.8$).



New ramapithecines and *Pliopithecus* from the Lower Pliocene of Rudabánya in north-eastern Hungary

THE increasing interest of specialists in problems of human evolution has involved not only the final phases, which are well documented but not satisfactorily settled, but also the critical examination of earlier phases. For the latter, however, researches rely mainly on the Indian *Ramapithecus*¹ and the East African *Kenyapithecus*² maxillary fragments, while almost entirely neglecting the corresponding European materials. The reason for this may be that the European finds were scattered specimens, or insufficiently described and therefore very difficult to evaluate. In addition, they are described under too many, probably superfluous, names.

To fill this gap we here provide some preliminary data concerning recently found corresponding materials from Hungary. At the same time, however, we emphasise that for a more complete survey of the whole problem it will also be necessary to take the important fossil anthropoid materials from Spain³ and those found recently in the Near^{4,5} and Middle East.

There were two earlier finds in the Carpathian Basin, in East Alpine localities: Dévény-ujfalu (now Devinska Nova Vés, Czechoslovakia)⁶⁻⁹, and St Stephan in Southern Austria¹⁰. A

Table 1 Cranial and dental hominoid remains from Rudabánya

Specimen no.	Year collected	Specimen	Collected by*	Determination
Rud-1	1967	Mandibular ramus sin. (with P ₄ -M ₃) (Fig. 1)	G.H.	Rud. hung. (type)
Rud-2	1969	Damaged mandible (with C-M ₃ dext. and sin.)	G.H.	Rud. hung.
Rud-3	1971	M ₃ dext.	G.H.	
Rud-4	1971	C sup. sin. (♂)	G.H.	Pli. her.
Rud-5	1971	P ₃ dext. (germ)	G.H.	
Rud-6	1972	M ¹ sin.	I.V.	Rud. hung.
Rud-7	1972	Maxillary fragment (with P ⁴ -M ²) (Fig. 4)	G.H.	Bod. alt. (type)
Rud-8	1972	C sup. sin. (♀)	G.H.	Pli. her.
Rud-9	1972	Fragments of mand. dext. and sin. (with C, D ₄ , P ₄ -M ₂ dext. and M ₁ -M ₃ sin.) (Fig. 6)	G.H.	Pli. her. (type)
Rud-10	1973	P ³ sin.	G.H.	
Rud-11	1974	M ₂ dext. (germ)	G.H.	
Rud-12	1974	Left maxillary and palate (with I ¹ , C-M ¹) (Fig. 2)	J.H.	Rug. hung.
Rud-13	1974	M ² sin.	J.H.	Rud. hung.
Rud-14	1974	Mandibular fragments (with I ₁ -P ₄ , M ₁ -M ₂ dext. and I ₁ -C, P ₄ -M ₂ sin.) (Fig. 5)	G.H.	Bod. alt.
Rud-15	1974	Left maxillary (with I ¹ -M ²) and fragments of right one (with I ¹ -M ²)	I.P.	Rud. hung.
Rud-16	1974	M ₃ dext.	G.H.	Rud. hung.
Rud-17	1974	Corpus of left mandible (with C-M ₃) and fragments of left mandible (with I ₂ -M ₃) (Fig. 3)	K.B.	Rud. hung.
Rud-18	1974	M ³ sin.	G.H.	Bod. alt.
Rud-19	1975	M ₃ sin.	G.H.	Rud. hung.
Rud-20	1975	C sup. dext. (♀)	G.H.	Pli. her.

Numbers are not Hungarian Geol. Survey inventory numbers.

*Names of collectors are: G.H., Gábor Hernyák; I.V., István Vörös; J.H., János Harnos; I.P., István Pálfalvy; K.B., Klára Beőreöndy.

(*Pliopithecus hernyáki* Kretzoi^{11,12}). The other two are pongo-hominids: *Rudapithecus hungaricus* Kretzoi 1967¹³⁻¹⁸ and *Bodvapiithecus altipalatus* Kretzoi 1974^{11,12}. Brief information about the hominid materials is given in Tables 1 and 2.

The main characteristics of the new *Pliopithecus* species and the two pongo-hominids respectively are the following:

1. *Pliopithecus hernyáki* Kretzoi 1974^{11,12}. A species of the genus *Pliopithecus* s.l. of big size, with rounded pentagonal P₄, strong labial cingulum on the molars and well developed posterior fossa (triangular in M₃) with disto-lingual notch. The crest on the axial side of the protoconid (where it should meet the corresponding crest of the metaconid) is missing. Dimensions: D₄ 8.2-5.5 mm, P₄ 5.9-6.4 mm, M₁ 7.8-6.5 mm, M₂ 8.5-6.8 mm, M₃ 10.2-6.3 mm. Holotype: Rud-9 (Fig. 6)—The Rudabánya species seems to represent an independent superspecific taxon beside the described subgenera *Pliopithecus* s.str., *Epipliopithecus* and *Plesiopliopithecus*, for which the name *Anapithecus* n.g. is suggested.

2. *Rudapithecus hungaricus* Kretzoi 1967¹³⁻¹⁵. A hominoid of slight build with a short face; high symphyseal, parasagittal, section of premaxillaries; wide incisive canal; flat palate; subparabolic dental arc; relatively small I¹; premolars subequal in size; low, brachyodont postcanines without cingula. Length of M₁-M₃: 29.5-31.0 mm. Holotype: Rud-1 (Fig. 1a, b), paratype: Rud-12 (Fig. 2a-d).

3. *Bodvapiithecus altipalatus* Kretzoi 1974^{11,12}. A robust form of bigger size than *Rudapithecus*; high vaulted palate; relatively hypsodont post canines with marked cingula, roughly sculptured, thick enamel surface. The dimensions of M₁-M₃ are about 37-38 mm. Holotype: Rud-7 (Fig. 4a-c), paratype: Rud-14 (Fig. 5 a, b).

There are some significant differences between the holotypes of *Ramapithecus brevirostris* and *Rudapithecus hungaricus*. *Ramapithecus* has a high, vaulted palate and the cross section

of the alveolar axis of the upper canine is longer and turned outward antero-laterally. (Former authors, like G. Lewis, failed to realise that the deformed alveolus of the canine gives the false impression of an abbreviated cross section.) *Ramapithecus* has a more antero-laterally situated I² so its dental arc is not subparabolic but more broadened in the frontal part. This means that *Ramapithecus brevirostris* (not identified with *Dryopithecus punjabicus* in my opinion), in spite of its younger geological age, is in some respects more ape-like, or more primitive than *Rudapithecus*, and represents a different line of hominid evolution.

Kenyapithecus with its flat palate, deep preorbital fossa, roughly enamelled and cingulated, higher-built postcanine dentition and somewhat bigger size, differs significantly from both *Rudapithecus* and *Ramapithecus*, in spite of the fact that most recent authors consider it identical to the Siwalik speci-

Fig. 2 *Rudapithecus hungaricus* Kretzoi, left upper jaw fragment, Rud-12. a, Lateral; b, occlusal; c, frontal; d, aboral view (×1).

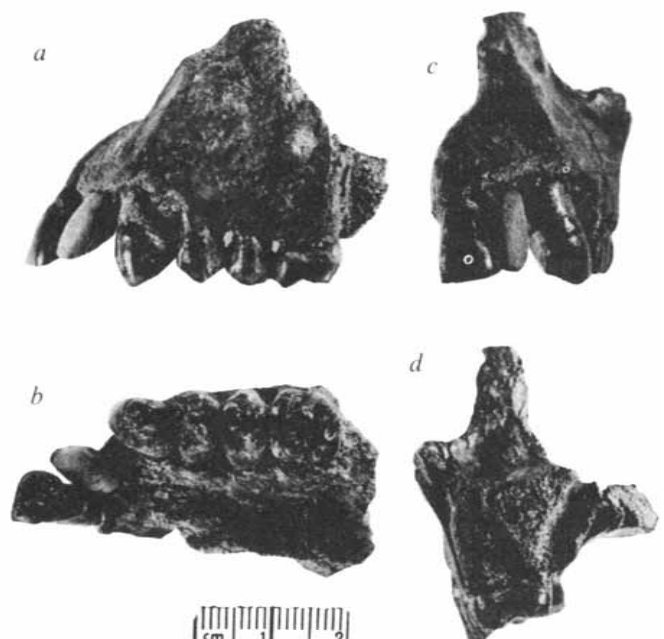


Table 2 Postcranial hominoid remains from Rudabánya

No.	Specimen	Determination
1	Distal fragment of left humerus	? <i>Rudapithecus</i>
2	Caput of the left femur	? <i>Rudapithecus</i>
3	Distal end of left femur	? <i>Rudapithecus</i>
4	Proximal end of left tibia	? <i>Rudapithecus</i>
	(most probably of the same specimen)	
5	Left astragalus, without head	? <i>Rudapithecus</i>
6	Distal end of metapodial	
7	Distal part of phal. I	? <i>Rudapithecus</i>
8-9	Medial fragments of phal. I	? <i>Rudapithecus</i>
10-11	Distal parts of phal. II	? <i>Rudapithecus</i>
12	Distal two-thirds of phal. I	? <i>Bodvapiithecus</i>
13	Distal part of phal. I	? <i>Bodvapiithecus</i>
14	Phal. I, proximal end missing	<i>Pliopithecus</i>
15	Distal half of phal. I	<i>Pliopithecus</i>
16	Diaphysis of phal. I	<i>Pliopithecus</i>
17	Medial fragment of phal. I	<i>Pliopithecus</i>
18	Proximal end of phal. III	? <i>Pliopithecus</i>



Fig. 3 *Rudapithecus hungaricus* Kretzoi, right mandibular corpus, Rud-17. a, Lateral; b, occlusal view ($\times 1.4$).

men. In addition to the differences between *Kenyapithecus* and *Ramapithecus*, *Rudapithecus* has, as distinct from *Ramapithecus*, an even flatter palate, in this respect resembling *Kenyapithecus*.

When comparing our material with anthropoid material considered to be such on the basis of their measurements, the situation is complicated by their poor preservation. We, therefore,



Fig. 4 *Bodvapihthecus altipalatus* Kretzoi right maxillary fragment, Rud-7 (type). a, Occlusal; b, aboral; c, lateral view ($\times 1.4$).

limit our comparison to the remark that the femur of the *Paidopithecus rhenanus*—reconstructed by mistake to about one inch longer than its real size—cannot actually differ greatly from *Rudapithecus*. It is not impossible, however, that the much-debated femur of *Paidopithecus rhenanus* is closer in size to the forms represented by the M_3 named *Neopithecus brancoi* or the small molar referred to as '*Rhenopithecus*'. The scantiness of the *Hispanopithecus laietanus* and *Udabnopithecus garedziensis* materials make satisfactory comparison impossible. Apart from patterns common to all ramapithecines, or representing only the general trend of hominisation, there is no close relationship between *Bodvapihthecus* and the forms mentioned above, although the construction of its palate resembles *Ramapithecus* and its general dental trend *Kenyapithecus*. The two recently described finds from the Balkans (*Grecopithecus freybergi*⁴ and

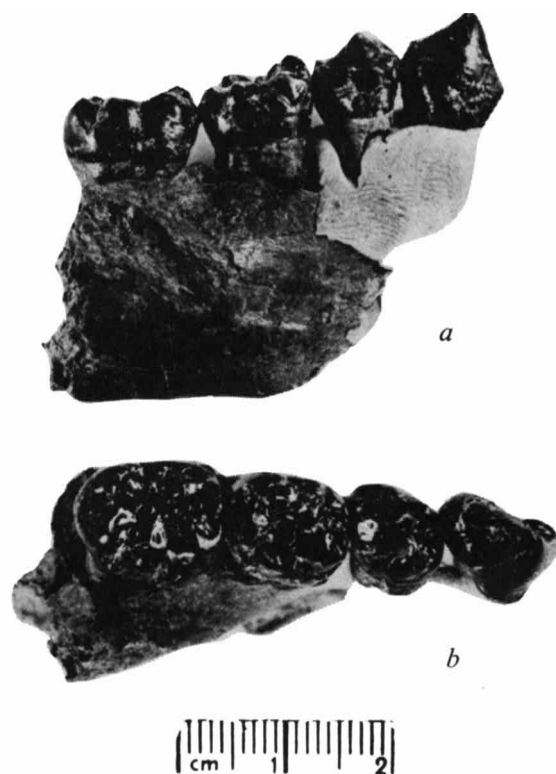


Fig. 5 *Bodvapihthecus altipalatus* Kretzoi, right mandibular fragment, Rud-14. a, Lateral; b occlusal view ($\times 1.5$).

*Dryopithecus macedoniensis*⁵) are larger and thus differ from both ramapithecines found in Rudabánya. Finally, *Dryopithecus keiyuanensis*, *Sivapithecus africanus*, *S. occidentalis* and *Rahonapithecus sabadellensis* ought to be mentioned and compared with our material as pongo-hominid forms tending towards hominisation. Because of their fragmentary representation or even lack of description, however, they are not really suitable for comparison. The same is also true of the remains described under the names of, for example, *Dryopithecus punjabicus*, and *Bramapithecus thorpei*. In these circumstances the most useful solution is to retain the identifications derived from well-preserved materials, suitable for further comparison, until the more correct names can be more precisely defined, hopefully, on the basis of further and richer material.

Although the taxonomic status of *Rudapithecus* is not influenced by its relationship to the forms representing the later phase of hominisation, it should be mentioned. The facial structure of *Rudapithecus* evinces a trend of hominisation which indicates a straight line of evolution through forms like *Pithec-anthropus modjokertensis* (restricted to the Sangiran IV maxillary) towards *Homo*. This seems to make it probable that australo-



Fig. 6 *Pliopithecus heryáki* Kretzoi left mandibular fragment with D₄-M₃, Rud-9 (type). Occlusal view ($\times 2$).

pithecines represent a close side branch with their independent development (decrease in size of front teeth, enlargement of molars, and so on), in many respects surpassing *Homo sapiens*, but not finally achieving the *Homo* level of evolution.

On the basis of the preliminary examination of the pongo-hominids of Rudabánya we can state the following conclusions: (1) Hominisation did not originate in certain isolated and more or less small tropical-subtropical gene pools, but was an evolutionary trend covering the whole Afro-Eurasian faunal radiation with its pongo-hominids. (2) Extant anthropoid apes, on the other hand, achieved their present non-human phase and trend of evolution as a result of a more or less extreme forest dwelling specialisation developed in certain isolated, tropical forest areas in Central Africa and south-east Asia. (3) Anthropoid evolution tending towards hominisation obviously developed on different levels, in numerous parallel, more or less 'human' branches which evolved so many parallel patterns during the process that it is not easy at present to find among them the single line which leads to the evolutionary development of *Homo sapiens*—and perhaps this is not even our primary concern at the moment, being more interested in collecting basic data. (4) The difficulties of identifying the previous, not satisfactorily defined, taxa make it necessary to use for the time being, the well-definable, later established taxonomic names.

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Tilt aftereffects with subjective contours

CELLS in the visual system respond well to spatial discontinuities in luminance (edges), but they are poorly activated by diffuse light¹. It has been proposed² that the selective response of edge-detectors to properties such as orientation and motion underlies form and pattern perception. In these terms, local discontinuity within the visual display is a necessary condition for perception of an edge. There are, however, anomalous contour effects³ (see examples in Fig. 1a and 1b) in which edges are clearly visible at sites where the visual stimulus is homogeneous. Such edges have been called subjective or cognitive contours on the supposition that cues to apparent depth within the display lead the observer to infer that he is seeing one plane located in front of, and interrupting, another plane^{4,5}. In these terms, "Since every plane must have an edge, the bounding contour is supplied by the perceptual system . . . a subjective contour is simply the edge of a subjective plane, and a

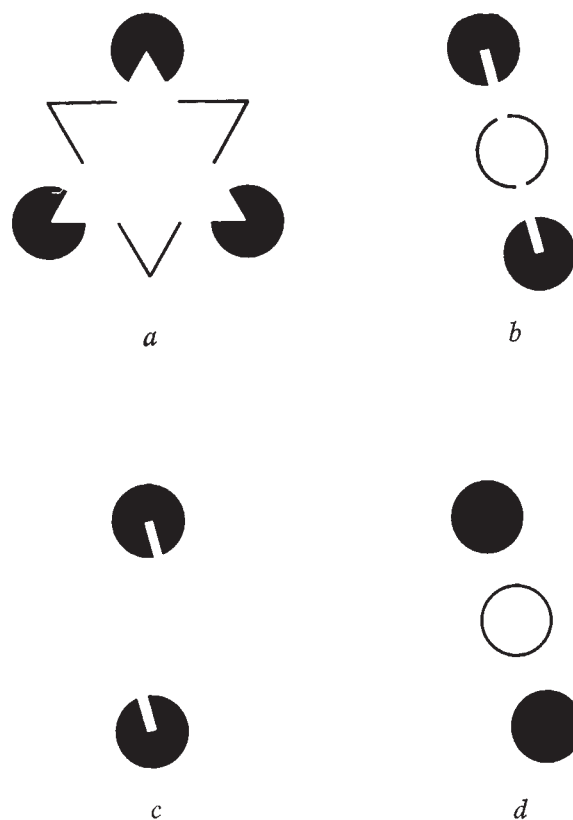


Fig. 1 Edges are visible within (a) and (b) in the absence of local luminance discontinuity. (b) was used as the subjective contour stimulus in the experiment. Test measures were also obtained following inspection of (c) and (d); these displays were used as control conditions in that they do not yield subjective contours but have some real contours in common with (b).

subjective plane is a surface which ought to be present on the basis of available depth cues, but is not except in the mind of the perceiver."

We report that the tilt aftereffect, in which a vertical line appears displaced clockwise after exposure to an anticlockwise tilted line, can be induced with subjective contours as well as with edges defined in terms of luminance discontinuity. In addition, exposure to a real contour results in a tilt aftereffect when the orientation of a subjective contour is subsequently judged, and vice versa. As the tilt aftereffect is generally attributed⁷ to adaptation of neural detectors which are tuned to contour orientation, these results raise the question of whether the perception of subjective contours has a basis at least in part in the activity of sensory feature detectors.

The experiment established the extent to which a test contour appeared displaced from vertical as a consequence of exposure to contours tilted 0°, 15°, 30°, 45° or 60° anticlockwise from vertical. This range of values was used because of evidence that the size of the tilt aftereffect depends on the difference in orientation between the inspection and test lines⁸. Aftereffects were measured for four conditions: inspection with real contour, test with real contour (R-R); inspect real contour, test subjective contour (R-S); inspect subjective contour, test real contour (S-R); inspect subjective contour, test subjective contour (S-S). The real contour, a white bar on a black ground, subtended either 4°36' $\times$ 12' (inspection stimulus) or 3°4' $\times$ 12' (test stimulus). The display shown in Fig. 1b was used to induce the subjective contour, which appeared in inspection and test conditions as a white bar subtending 4°36' by 12'. The top and bottom sections of this bar were

correlated with real contours, but the centre section subtending 3.4° was visible in the absence of local luminance discontinuity.

On each trial a centrally located fixation point was shown for 2 s, then the inspection bar for 1 s, and finally the test bar for 100 ms. The observer's task was to report whether the test bar appeared tilted clockwise or anticlockwise from vertical; the use of other response categories was not permitted. The orientation of the test bar was varied over trials by a double-random staircase with 1° steps. For each of the four observers eight staircases were completed at the five inspection orientations for the four stimulus conditions. The order of testing conditions across observers was controlled by a Latin square, and orientation sequences were varied randomly between observers and conditions.

Figure 2 shows, for each stimulus condition, the mean orientation (displacement from true vertical) at which there was equal likelihood that the test bar appeared tilted clockwise and anticlockwise. The size of the aftereffect is given by the difference between values obtained when the inspection bar was vertical and those found after exposure to a tilted bar. An analysis of variance based on difference scores calculated for each observer showed that test judgments varied significantly as a function of the orientation of the inspection bar ($F(3, 9) = 32.30$, $P < 0.01$). The trends are consistent with other data⁸ on the orientational selectivity of the tilt aftereffect. The size of the aftereffect was independent of whether the inspection or test bars were formed by real or subjective contours ($F(3, 9) = 2.68$, $P > 0.05$), and the interaction between the orientation of the inspection bar and the type of display was also insignificant ($F(9, 27) = 0.86$, $P > 0.05$).

The possibility needs to be considered that judgments for condition S-S, S-R and R-S were under the control of the local luminance discontinuities within the display used to induce subjective contours instead of the spatial properties of the subjective contours themselves. It should be recognised that these two variables cannot be studied in isolation; the presence of real contour information is a necessary condition for the induction of subjective con-

tours. It is possible, however, to measure the apparent tilt of real and subjective test bars after inspection of patterns (such as those shown in Fig. 1c and d) that do not yield subjective contours although they have some real contours in common with Fig. 1b. Measures obtained in such control conditions differed significantly from the largest aftereffects obtained for conditions S-S, S-R and R-S. The four subjects also judged the real and subjective test bars after adaptation to Fig. 1b without the broken circle at the centre, the broken circle alone, Fig. 1b with a complete circle at the centre and eight dots arranged as a bar. Mean displacements ranged from $+0.41^\circ$ to $+1.22^\circ$; all values were significantly less than the largest aftereffects shown in Fig. 2. These results can be treated as weak main effects rather than as control data in that the adaptation displays other than the eight-dot stimulus yielded slight subjective contours.

Our results indicate that the tilt aftereffect can be generated with subjective as well as with real contours. The orientational selectivity of the aftereffect is similar for the two types of stimulus. In addition, the apparent orientation of an edge that is correlated with luminance discontinuity can be shifted by previous exposure to a phenomenal edge at a location within the display that is uniform in luminance. In recent accounts neural edge-detectors have been implicated in the perception of real contours, whereas subjective contours have been treated as products of cognitive or inferential operations. Although data indicate^{9,10} that subjective contours cannot be explained within a simple sensory theory, our results suggest that it is premature to attribute the perception of real and subjective contours to fundamentally different processes.

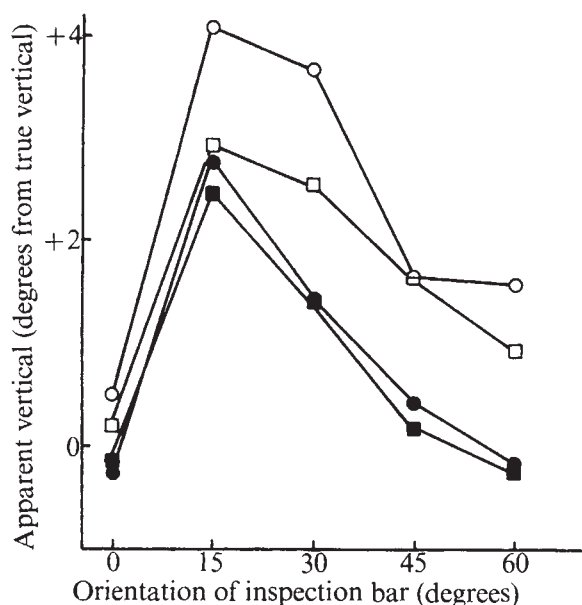
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Fig. 2 Mean location at which a test bar appeared vertical after exposure to an inspection bar tilted from 0 to 60° anticlockwise. Measures are expressed relative to true vertical, with clockwise displacements given negative scores and anticlockwise displacements positive scores. Values are shown separately for the four display conditions described in the text. ●, R-R; □, R-S; ■, S-R; ○, S-S.



Ambiguous cognitive contours

CONTOURS perceived in the absence of physical gradients of stimulation have been called cognitive contours¹, subjective contours^{2,3}, contours without gradients⁴, anomalous contours⁵ and quasi-perceptive margins⁶. The phenomenon is produced by placing inducing elements in special arrangements on a surface of uniform luminosity, as in Fig. 1. A whiter-than-white triangle can be seen overlying three black disks and a white background. The apparent brightness difference and the perceived contours extending between the disks are illusory. Gregory¹ discusses two paradigms, the cognitive and the physiological, which have been used to explain these contours. We introduce here two configurations which support the cognitive paradigm.

Figure 2 presents a subjective contour configuration which is perceptually ambiguous. Whereas Fig. 1 produces only one set of illusory contours (those corresponding to the 'sides' of the triangle), the 'ship's wheel' configuration can be perceptually organised in various ways, each resulting in an arrangement of illusory contours unique to that organisation. Three objects can be differentiated in the wheel-shaped structure of the figure; +, × and ○. These stratify in depth in a specific manner for any particular organisation of the figure. For example, the + can be seen overlying the ×, which in turn overlies the ○. The

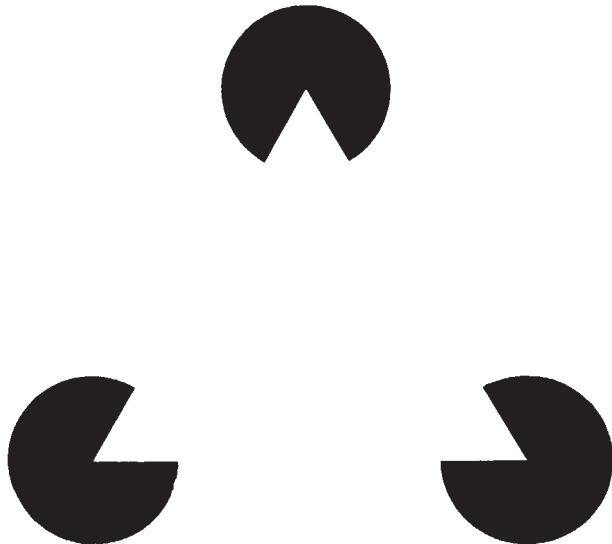


Fig. 1 A standard subjective contour configuration: a phenomenally complete triangle is perceived overlaying three black disks.

bars of the + and the $\times$ are seen to pass over the $\circ$, with illusory contours extending across it. The relative positions of these objects may spontaneously reverse, with the $\circ$ now appearing on top. Curved illusory contours demarcating the phenomenal borders of the $\circ$ are then seen extending across the bars of the + and $\times$. Alternatively, the + may be seen overlying the $\circ$, and both of these overlying the $\times$. In this case, the straight subjective contours of the + are seen passing over the $\circ$, and the curved subjective contours of the latter are seen passing over the $\times$. Clearly, there are as many possible arrangements of illusory contours as there are possible alternative organisations of Fig. 2, and the contours will be observed to 'shift' about the display during the transition from one organisation to the next.

Figure 3 presents another ambiguous subjective contour display. Illusory contours corresponding to the inside corners of a six-pointed 'star' may be seen extending between adjacent disks. Alternatively, an upright triangle can be seen overlying an iden-

Fig. 2 The ship's wheel configuration: the +, $\times$ and $\circ$ alternate in apparent depth over time, thus causing the subjective contours corresponding to the phenomenal 'edges' of these objects to shift about the display. Subjective contours are not seen if the central white region is perceived as a completely solid object localised entirely in one plane (for example, a ship's wheel).



tical, but inverted, triangle. In this case, the subjective contours of the upright triangle are seen to proceed in straight lines, cutting across the 'corners' of the inverted triangle as they do so. If the relative positions of the triangles reverse, the subjective contours of the inverted triangle will now seem to cut across the corners of the upright triangle.

Corresponding brightness effects are associated with the alternative organisations of Figs 2 and 3. Objects which are seen as overlying other objects (or backgrounds) are perceived as somewhat brighter. For example, when the $\circ$ is seen on top in Fig. 2 it seems somewhat brighter than the + and $\times$. Similarly, when the upright triangle is topmost in Fig. 3, it seems brighter than the inverted triangle which it overlies, and the latter, in turn, seems brighter than the background. When the perceptual organisation reverses the relative positions of the objects, the brightness relations reverse as well.

Since Figs 2 and 3 consist only of specially arranged inducing elements on white surfaces otherwise perfectly uniform in luminosity, the perceived contours and brightness effects are, of

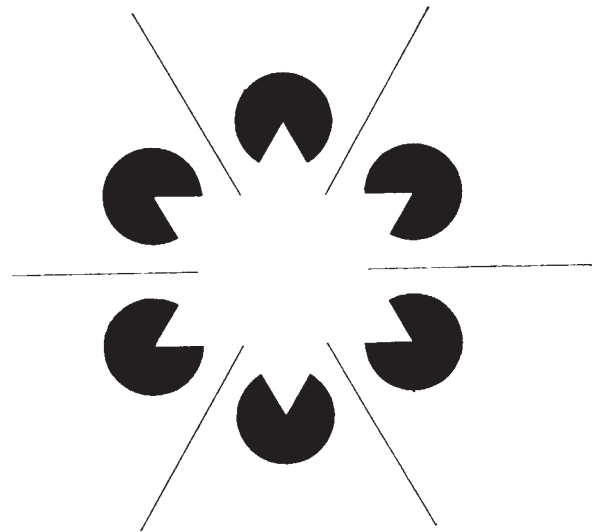


Fig. 3 The star configuration: either a six-pointed star or two superimposed triangles (with one inverted) may be seen. The perceived location of the illusory contours depends on the prevailing perceptual organisation.

course, illusory. The important point, however, is that different perceptual organisations of the same figure generate different illusory effects. Consequently, any theory which attempts to account for subjective contours must accommodate the fact that these phenomena are not totally stimulus-bound: that is, what is seen in Figs 2 or 3 cannot be predicted simply from a knowledge of the luminosity distribution of the physical array. Since physiologically oriented theories of subjective contours are often stimulus-bound in this sense⁷, they cannot explain our findings. The view that perception is a process of hypothesis formation in which 'object hypotheses' are selected by, but go beyond, sensory data⁸ is, however, consistent with these findings. If a configuration presents sensory data which can support multiple object hypotheses (Figs 2 and 3), then shifting illusory contours would be predictable as the result of alternative hypotheses being sequentially tested and applied to the ambiguous array.

There are two major limitations of the cognitive theory. First, the theory cannot predict which specific object hypothesis, of many possible, will be selected in a given instance. Second, the cognitive theory has not attempted to explain the brightness differences which are consistently observed in subjective contour configurations. Competing theories often start by explaining the brightness differences first, and then derive the contours from the apparent brightness gradients which result^{7,9}. Cogni-

tive theory holds that it is the formation of object hypotheses going beyond sensory data which gives rise to subjective contours, so it must be shown that the associated brightness effects arise concurrently with, or subsequent to, the formation of the contours, rather than casually preceding them. We would argue that the apparent brightness differences derive from a classic figure-ground phenomenon: that is, a figure will generally seem brighter or more intense than a background of equal reflectance value¹⁰. In so far as the central white area in Fig. 1 is conferred the status of an object (triangle), it will necessarily be seen as figure overlying a background. Consequently, its brightness will be enhanced and a whiter-than-white triangle will be perceived. This would also account for the reversals in apparent brightness seen in Figs 2 and 3 which arise when the perceptual organisation reverses the relative positions of objects in the display. As one object becomes 'figure' relative to another by overlying it, it will also take on a phenomenally brighter or more intense appearance¹¹.

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Development of cat visual cortex following rotation of one eye

EVER since the first description of the optical inversion of images within the vertebrate eye, philosophers and psychologists have pondered the question of how animals interpret the directions of objects in space. The physiological discovery of retinotopic 'maps' in the brain (such that the retina is represented in an orderly array in the tectal¹ and cortical² visual areas) seemed to provide an answer to the question. Neurones in these visual centres have receptive fields that are restricted to a particular region of the retina and could therefore preserve information concerning the positions of objects in the visual world.

Humans show considerable ability to compensate for prism-induced displacement³, partial rotation⁴ and even complete inversion⁵ of the retinal image. The physiological mechanisms underlying this capacity are not understood. On the other hand, amphibians never adjust their orienting behaviour to adapt to physical rotation of the eye⁶. Indeed, if the optic nerve

is severed at the time of rotation, the fibres regenerate and reinnervate the contralateral optic tectum, re-establishing the original relationship between retina and tectum and thus an inverted representation of the visual world⁷. These experiments suggest that lower vertebrates have a rigidly specified mapping system to the contralateral optic tectum, which is uninfluenced by visual experience, whereas in higher mammals visuomotor behaviour is responsive to alteration of the visual input. In fact in kittens initial acquisition of visually co-ordinated behaviour, such as guided locomotion and guided



Fig. 1 The kitten (K131) whose right eye was surgically intorted by 109°.

reaching, requires visual experience with feedback from movement⁸.

We have developed a technique for torsional rotation of the cat's eye (without sectioning the optic nerve, since it will not regenerate). The results of this procedure have provided information about the plasticity of visual localisation in cats, and about the genetically specified restrictions on the properties of cortical cells.

In three kittens less than 3 weeks old, one kitten that had been kept in total darkness until 5½ weeks old, and one normal adult, the insertions of all the extraocular muscles of the right eye were divided and the whole globe gently intorted, taking care not to stress the optic nerve or the blood supply to the eye. In each case the eye remained in its new position without suturing, and the conjunctiva healed very rapidly. Within a few days partial muscle reattachment occurred and limited eye movements returned.

All animals were then housed in a colony room (illuminated for 18 h each day), with both eyes open. Several weeks later the animals' vibrissae were cut and their visuomotor coordination was tested, using diffusing contact lenses to cover each eye in turn. Their behaviour was entirely normal through the non-rotated left eye and Table 1 summarises tests of their visual abilities through the rotated eye alone.

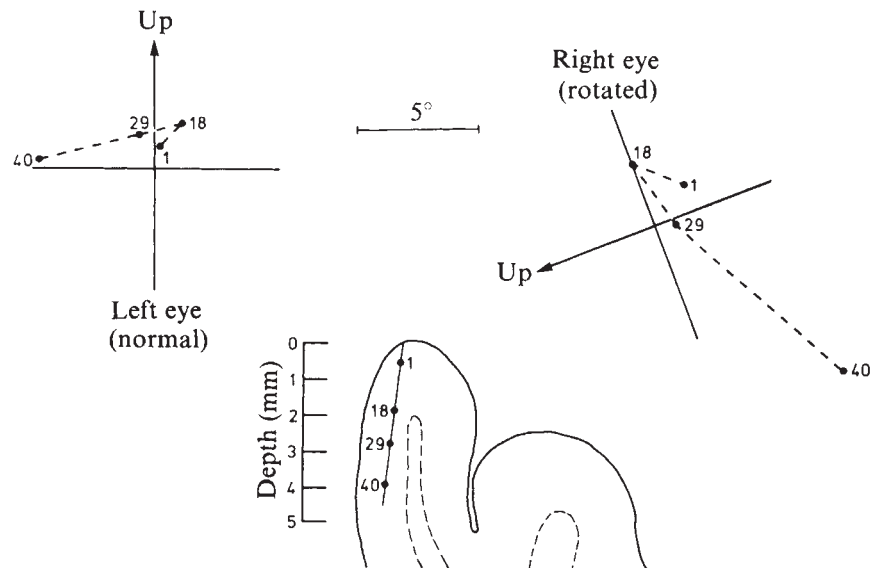
In all but one of these animals, with the normal eye occluded, head and eye movements could be elicited by presenting a small, high contrast object against a homogeneous background (visual following). Except in K108, the animal with the smallest amount of rotation (30°), however, the movements

Table 1 History of the animals and visual abilities using the rotated eye alone

Animal	Age at rotation	Age at recording	Angle of intorsion of right eye	Visual following	Visual behaviour using the rotated eye "Triggered" visual placing	Visually guided reaching	Guided locomotion
K108	11 d	20½ weeks	30°	+	+	+	—
K126	21 d	19 weeks	72°	+	+	—	—
K140	Adult	5 weeks later	106°	+	+	—	—
K131	16 d	17 weeks	109°	+	+	—	—
K137	38 d (previously dark-reared)	11 weeks	115°	—	+	—	—

+, Visual behaviour present; —, Visual behaviour absent.

Fig. 2 Preservation of approximate retinotopic projection in the kitten (K131) shown in Fig. 1, whose right eye had been intorted by 109° . The electrode penetration through the visual cortex of the right hemisphere is reconstructed in the lower half of the figure, and the locations of four binocularly driven cells are marked on the track. Above are diagrams of the positions of the receptive fields of these cells, as they appeared on the screen in front of the animal. The centre of each field is marked $\bullet$ and they are joined, in series, with interrupted lines. The projections in space of the true retinal vertical and horizontal meridians are drawn as solid lines, intersecting at the projection of the area centralis. The upper visual field of each eye (the field of the inferior retina) is marked $\rightarrow$, labelled 'up', on the projection of the vertical meridian.



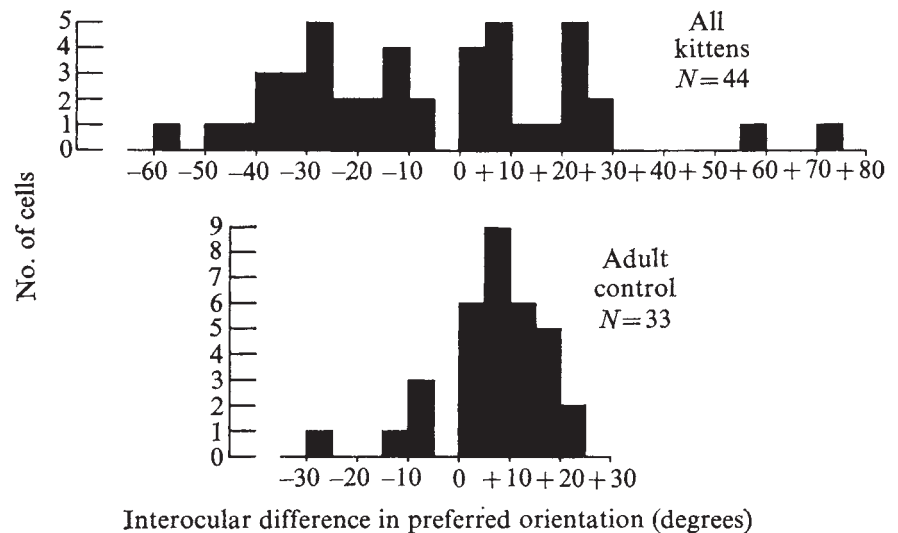
were rather jerky, unlike the smooth tracking seen in normal cats. Although the gross sequence was usually in the correct direction, the individual jerky movements may have contained inappropriate components, which were corrected by later movements. This unusual strategy of tracking deserves further study using more exact techniques for observing eye and head movement.

When lowered towards a broad horizontal surface, every cat extended its forelimbs downwards at an appropriate

of rotation, the responses were not very accurately directed in space. The lack of complete visuomotor compensation is, perhaps, not surprising in view of not only the rotation itself but also the relative immobility of the operated eye and the conflicting information from the other eye.

Behavioural compensation might be more complete with a longer interval between operation and testing, or if the normal eye were closed from the time of rotation. Both of these expectations seem to be confirmed by more recent experiments: two

Fig. 3 Analysis of the interocular similarity of preferred orientation for 44 binocular cells from kittens with eye rotation and 33 from the adult animal. The receptive field orientation was measured on retinal coordinates¹³ and, for each unit, the algebraic difference between the preferred orientations in the two eyes gave the value plotted on the abscissae of the histograms. Cells with identical orientation preference on the two retinæ have a value of zero. The distribution for the kittens, which had not had previous prolonged normal binocular vision, is significantly broader than that for the adult control ($P < 0.001$, F test, d.f. 43,32). The variance is not accounted for merely by pooling of data from several animals, since individual kittens showed very wide variability in the differences in preferred orientation.



distance from the surface ('triggered' visual placing). In normal cats such triggered extension is elicited only by approach to a horizontal surface: however, except for K108, movement of the limbs in the same direction sometimes also occurred if these animals were brought near a vertical surface.

K108 showed visually-guided reaching. When tested with an interrupted surface of the type introduced by Hein and Held⁹, this animal reached accurately for the prong, and avoided the gaps between prongs. In contrast, the animals with larger angles of rotation were equally likely to place the paw on a prong or in a gap. Finally, none of the animals managed consistently to avoid objects when moving through an obstacle course (guided locomotion). The results of eye rotation in the adult cat (K140) were not obviously different from those in kittens with comparable angles of rotation.

Thus the rotated eye was able to mediate behaviour to visual input, but, except for the animal with the smallest angle

cats tested more than a year after 90° rotation are much more adept when using the rotated eye alone than any of the cats in the present study. A further animal with a 90° rotation that had the lids of the other eye sutured showed the best behavioural recovery of all, having quite reliable tracking movements, excellent performance on the obstacle course and accurate visually guided reaching.

Immediately after behavioural testing, we used conventional neurophysiological techniques to record from single neurones in the visual cortex¹⁰. Each animal was initially anaesthetised with fluothane and then Brietal sodium, immobilised with an intravenous infusion of Flaxedil and then artificially ventilated with 80% nitrous oxide. The eyes were optically corrected with contact lenses, spectacle lenses and 3-mm artificial pupils. The receptive fields of cortical cells were plotted by back-projecting stimuli such as spots and lines on to a tangent screen in front of the cat with each eye occluded in turn. The

vast majority of neurones were orientation selective, just as in the visual cortex of a normal cat¹¹. Only 5 out of 147 cells in the kittens and 0 out of 44 cells in the adult were unresponsive to our visual stimuli.

Photographs of the slit-shaped pupils taken before the preparation (see Fig. 1) and after paralysis enabled us not only to measure the surgically induced intorsion of the right eye (assuming that the two pupils should have been symmetrically orientated), but also to assess any further rotations of the eyes induced by paralysis. Thus we were able to relate the position and preferred orientation of each receptive field to the corrected horizontal and vertical meridians, on true retinal coordinates.

Most cells in our adult cat, as in normal animals¹¹, were excited by stimuli shown to either eye (35 out of 44 cells). Not surprisingly, however, the majority of visually responsive neurones in the younger animals (95 out of 142) were monocularly driven, just as in kittens with a surgically induced lateral deviation of the eyes¹².

The retinotopic map of the rotated right eye was virtually unchanged. Each region of the cortex represented approximately corresponding areas of the two retinæ, just as it had before rotation. Figure 2 shows the results for one animal (K131) whose right eye had been intorted by 109°. The histologically reconstructed microelectrode penetration is drawn below, and above it, just as they appeared on the screen, are the positions of the receptive fields of four binocularly driven neurones recorded at the points marked by filled circles on the reconstructed electrode track.

The projections on the screen of true horizontal and vertical retinal coordinates, intersecting at the area centralis, are shown for each eye. For this sequence of neurones the receptive fields in both eyes shifted from the vertical meridian, slightly into the field of the contralateral hemiretina and then far out into the field of the ipsilateral hemiretina, typical of a penetration that starts in area 18 and moves deep into area 17. The other units recorded in this penetration, which were mainly monocular, had receptive fields that fitted this general progression. In every animal we observed this preservation of the normal retinotopic mapping from the rotated eye, and recently we have confirmed the finding in another kitten with a sample of receptive fields extending more than 30° into the peripheral retina.

Binocular cells in these kittens tended to have similar receptive field properties on the two retinæ in spite of the fact that there was no possibility of regular correlated binocular input after the rotation. In particular, we measured the preferred orientations on true retinal coordinates and Fig. 3 shows histograms of the differences between the two receptive fields for all binocular neurones that had clear orientation selectivity in both eyes (44 cells from the kittens, 33 from the adult). Cells from the normal adult with eye rotation had very closely matched preferred orientations on the two retinæ just as in normal cats¹³. Cortical cells in the kittens (who had not experienced a prolonged period of binocular vision before eye rotation) also, on average, had similar orientation preferences on the two retinæ. The range of differences (approximately $\pm 70^\circ$) was, however, much greater than in normal animals.

Thus there seems to be a definite innate restriction on the mapping from retina to visual cortex and, to some extent, on the interocular similarity of preferred orientations of cortical cells. Certainly the visually controlled behaviour displayed by cats with one eye rotated cannot be attributed to a reorganisation of the relationships between retina and visual cortex.

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We have recently learned that Yinon, Auerbach, Blank and Friesenhausen¹⁴ have also found binocularity to be reduced in the visual cortex of cats after rotation of one eye.

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Light-induced formation of dense-core vesicles in rod photoreceptors in retina of *Xenopus laevis*

SEVERAL attempts have been made to correlate changes in the fine structure of synaptic regions of vertebrate photoreceptor cells with dark or light adaption^{1–5}. Synaptic vesicles in both rods and cones were reported to be reduced in size after prolonged exposure to darkness^{1,2}. Later workers^{3–5}, however, have not substantiated these claims. Mountford³ did not find any changes in vesicle populations in photoreceptors in response to changes in illumination; neither did Cragg⁴, although he did find that receptor terminals increased in width after prolonged periods in the dark, and decreased in width after only 3 min of exposure to daylight. Wagner⁵ showed in fish retina, that in animals kept under a constant day–night regime, the numbers of synaptic ribbons were reduced in fish fixed towards the end of the night period.

The upshot of this previous work is that there are no unequivocal descriptions of changes in vesicle structure which can be correlated with changes in illumination. We were therefore intrigued to find changes in the morphology of vesicles in the synaptic region of rods in the retina of *Xenopus*, brought about by exposure to continuous light. In the rods of toads kept in normal day–night conditions, or in constant darkness (up to 12 d), the synaptic vesicles surrounding the ribbons and in the neighbouring cytoplasm were all agranular (Fig. 1a). On the other hand, in animals kept under constant illumination (0.5 m distant from a

Table 1 This shows the mean % of dense-core vesicles in the synaptic vesicle population associated with synaptic ribbons relative to the period of exposure to continuous illumination

No. of days continuous light	Mean % of dense-core vesicles $\pm$ s.e.	P
4	24.55 $\pm$ 2.38	} ≤ 0.001 } 0.01
9	51.30 $\pm$ 3.36	
12	13.88 $\pm$ 3.29	

P is the probability between different treatments calculated by Student's *t* test. *P* > 0.05 not significant.

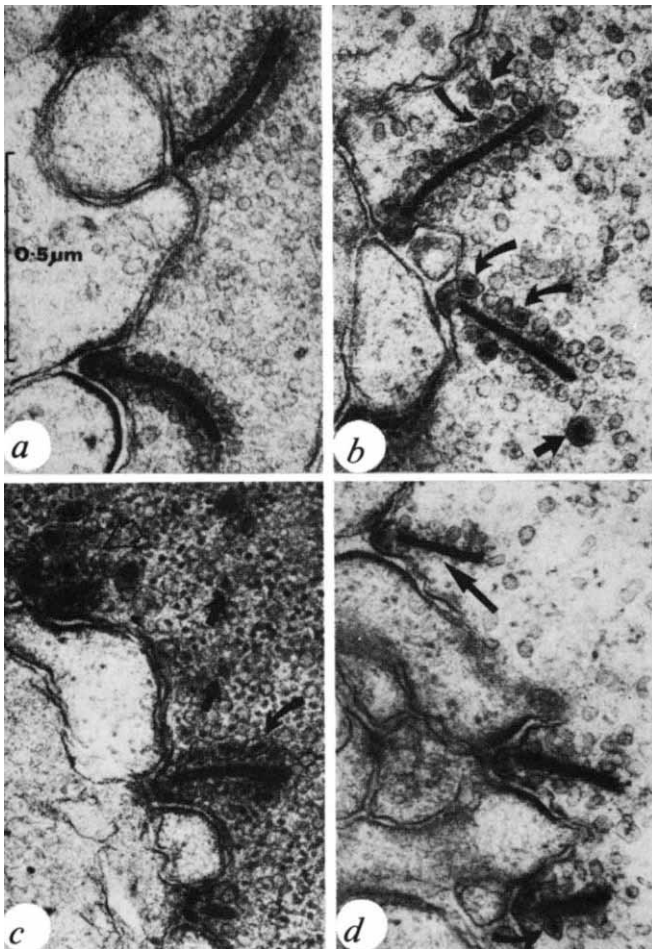


Fig. 1 Electron micrographs of rod pedicles of *Xenopus* retina from animals exposed to: *a*, day-night cycle; *b*, 4 d; *c*, 9 d; and *d*, 12 d continuous light. In (*a*) note that the three electron-opaque synaptic ribbons are surrounded by a cloud of exclusively agranular, synaptic vesicles. By contrast dense-core vesicles are present alongside the ribbons (curved arrows) in (*b*), (*c*) and (*d*) and in the cytoplasm (straight arrows) in (*b*) and (*c*). In (*c*), the open arrow indicates a clump of dense-core vesicles where the plane of section has just grazed a synaptic ribbon. In (*d*) note the absence of agranular vesicles from one side of a synaptic ribbon (straight arrow). Material prefixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in epoxy resin. Scale line applies to all parts of the figure.

240 V, 40 W pearl, tungsten bulb; illuminance = 86.08 lx) after 4 d dense-core vesicles were found alongside the synaptic ribbons and in the surrounding cytoplasm (Fig. 1*b*, Table 1). By 9 d, on average, just over half the vesicles associated with the ribbons had dense cores (Table 1), although in some sections through synaptic ribbons all the vesicles were the dense-core variety (Fig. 1*c*). Dense-core vesicles were also more common in the rod cytoplasm (Fig. 1*c*). Another interesting feature in many rods was the presence of what appeared to be glycogen particles (Fig. 1*c*). After 12 d in the light, the number of dense-core vesicles was drastically reduced compared with that after 9 d (Table 1). Moreover, some agranular vesicles were lost from around the ribbons (Fig. 1*d*). Our results therefore suggest that constant illumination over several days induces the rods to produce dense-core vesicles, but interestingly enough, no similar structural changes in vesicles have been seen in the cones.

What physiological activities could underlie the appearance of these dense-core vesicles in rods? It is possible that because vertebrate photoreceptors are hyperpolarised by light⁶, that this will reduce their rate of neurotransmitter release⁵. In these conditions it might be expected that the

transmitter stores would increase. Such an increase in the transmitter store may account for the appearance of the dense-core vesicles and for their progressive increase in number during 9 d. The drop in their number by 12 d is puzzling, but it could be attributed to metabolic changes within the rods associated with a cessation or reduction of transmitter synthesis. This could also account for the reduced numbers of agranular vesicles associated with some of the synaptic ribbons (Fig. 1*d*).

The identity of the neurotransmitter(s) in rods and cones is not known⁷, although amino acids, catecholamines and acetylcholine are all possible candidates for synaptic transmitters in the retina⁸. Turtle photoreceptors can synthesise acetylcholine⁹, and there are high levels of taurine in the photoreceptor cells of the frog retina¹⁰. The occurrence of dense-core vesicles in the rods of *Xenopus* appear to be inconsistent with the presence of either of these two substances, since this type of storage vesicle is thought to be indicative of indoleamine and catecholamine stores in neurones⁸. Recent fluorescent and electron autoradiographic studies have demonstrated that although monoamines are present in the retina, they do not reside in the rods and cones⁸. It has been reported, however, that a fluorescent amine is associated with the nuclear layer of rods and cones¹¹, although the authors discounted that the fluorescence was caused by monoamines known to be neurotransmitters, that is dopamine, adrenaline and 5-hydroxytryptamine. The presence of an unidentified amine in the photoreceptors while possibly supporting our structural evidence of dense-core vesicles in the rods, conflicts with our failure to demonstrate dense-core vesicles associated with the synaptic ribbons of cones. It could be argued, however, that because cones are less sensitive to light than rods, the level of illumination in our experiments was insufficient to cause a buildup of dense-core vesicles in this type of photoreceptor. If this is not the case then it at least raises the possibility that rods and cones have different neurotransmitters.

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Tolerance of *Lolium* hybrids to quantitative variation in nuclear DNA

THE genus *Lolium* contains six species, all diploids with 14 chromosomes. The chromosomes of the inbreeding species are larger than those of the outbreeders and carry about 30% more DNA¹. Although the range of variation in nuclear DNA amount is not large, in comparison with other genera of flowering plants², *Lolium* is exceptional in that crosses between diploid species with widely different amounts of DNA produce F₁ hybrids which set viable seed on backcrossing or, alternatively, by intercrossing to produce F₂s. Segregation of nuclear DNA amount among hybrid derivatives by independent chromosome assortment and crossing over at meiosis in the

F_1 makes feasible a genetic assay of the effects of the differential interspecific DNA component. This report describes the segregation of nuclear DNA amounts among F_2 progenies (seedlings 5–7 d old) from F_2 hybrids between *L. temulentum* with high DNA and *L. rigidum* with low DNA content. F_1 seeds were obtained by allowing the F_1 hybrids to intercross in isolation from foreign pollen. From the DNA distribution in F_2 it is possible to draw conclusions about the influence of the differential DNA component on gamete and zygote viability. The results are given in Fig. 1.

As the result of recombination in F_1 , DNA values range from approximately that of the low DNA parent to the high. Moreover, the distribution closely approaches and does not differ significantly from that of a normal curve. We conclude, in respect of the viability of gametes and of zygotes, toleration of a wide range of DNA amounts. Comparable quantitative DNA variation brought about by aneuploidy, that is by gain or loss of whole chromosomes, in *Lolium*⁴ as in most eukaryotes is the cause of severe genetic imbalance causing high mortality. We infer, therefore, that the 30% fraction of the DNA that distinguishes *L. temulentum* from *L. rigidum* is, genetically, of a substantially different character from that representative of whole chromosomes. Biochemical analyses in *Plethodon*⁵ have confirmed an earlier prediction⁶ that quantitative DNA

differences between related species involve mainly a particular and distinctive fraction of the nuclear DNA. The molecular distinctiveness finds a parallel in terms of genetic activity.

Growth and development of F_2 plants are normal in the sense that the variation in leaf production, shoot growth and flowering time lies within the range displayed by the parents. This is not to say, however, that the extra DNA in *L. temulentum* may be considered strictly inert. In this connection it will be observed that the mean nuclear DNA content of the F_2 progenies (23.6) is significantly lower ($P < 0.01$) than that of the mid-parent (F_1) value, 26.1. A possible explanation is that the development and viability of gametes or zygotes carrying mainly *L. temulentum* type chromosomes are at least partly dependent on the differential DNA fraction. Whether this interpretation is justified awaits additional evidence from more detailed assays.

The distribution of DNA amounts among the backcross progenies of *L. perenne* $\times$ *L. temulentum* given by Rees and Jones¹ leads to the same conclusions as those reported here. Above all, the evidence establishes, on a genetic rather than a biochemical basis, that the large scale variation in nuclear DNA amount among these eukaryote species involves a distinctive DNA component, distinguished by its relative inertness in comparison with that representative of whole chromosomes. In this respect there is an analogy with the DNA contributed by supernumerary B chromosomes⁵. Finally, since gametes and zygotes are tolerant of a considerable variation with respect to this nuclear DNA fraction it is the more surprising that the nuclear DNA amount within species of eukaryotes is so constant. The question arises as to whether the main restriction on quantitative change may, after all, lie more with the initiation of such change than with its consequences.

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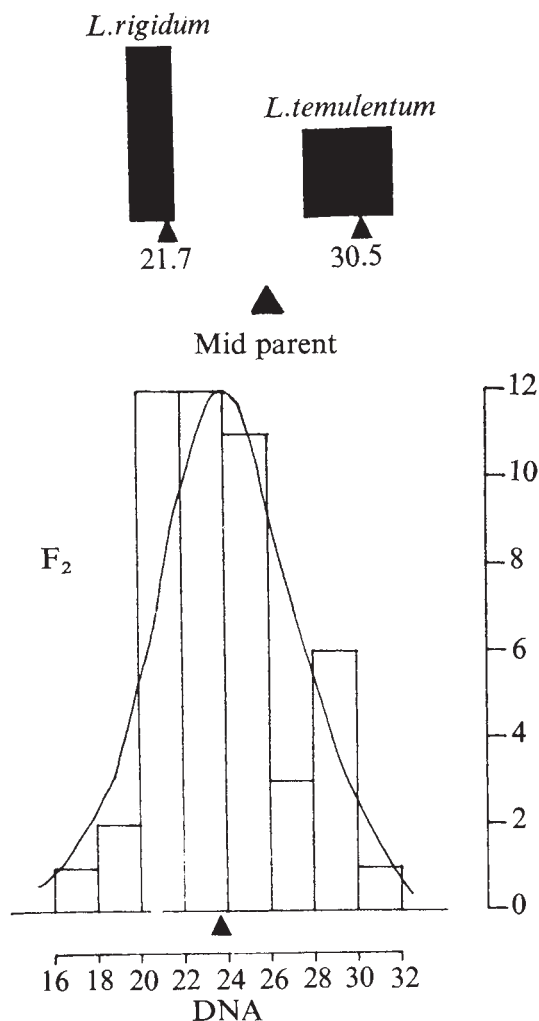
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Fig. 1 Nuclear DNA amounts (in arbitrary units) at G1 in *L. rigidum*, in *L. temulentum* and in 48 F_2 seedlings. Measurements were made on a microdensitometer (Vickers M85). Feulgen-stained root meristems were prepared by the method of McLeish and Sunderland³. Seedlings were handled in six batches of eight. A control (*L. temulentum*) was scored with each batch and adjustments were made to correct for minor fluctuations in readings between batches of slides. The theoretical normal curve is superimposed on the F_2 histogram.



Similarities and differences in latitudinal adaptation of two *Drosophila* sibling species

THE *melanogaster* subgroup in the *Drosophila* genus now includes six different species: four of them are found in tropical countries of the Ethiopian biogeographic region (*D. yakuba* Burla, *D. teissieri* Tsacas, *D. erecta* Tsacas and Lachaise, *D. mauritiana* Tsacas and David) whereas the two better known species, *D. melanogaster* and *D. simulans*, are widespread cosmopolitans. This geographical distribution is a strong argument for a tropical African origin of the subgroup¹ and it suggests that genetically only two species were sufficiently versatile for adaptation to northern and southern temperate climates.

Ecological observations, indicating that the two cosmopolitan species are linked to human activities², suggest that the species have been distributed by man during historical time; that they occupy similar ecological niches which are fairly constant all over the world; and that human transportation mixes the flies, resulting in a uniform genotypical composition throughout the range. Evidence is accumulating, however, that such suggestions may be incorrect and, consequently, that cosmopolitan species are indeed interesting for evolutionary studies³.

In *D. melanogaster*, linear latitudinal clines have been demonstrated for two quantitative polygenic traits (adult weight and female ovariole number) among temperate strains from Europe and North America, and tropical strains from equatorial Africa and America⁴. Preliminary studies suggested a similar phenomenon in *D. simulans*⁵. We have therefore undertaken a more extensive analysis. Results for 32 different strains of *D. simulans* (Fig. 1) are compared with the *D. melanogaster* data.

D. simulans is lighter and has fewer ovarioles than *D. melanogaster*; otherwise they are fairly similar. The fresh weight of females increases linearly with latitude and the slopes for the two species are otherwise not significantly different (the results for males (not shown) point to the same conclusion). For ovariole number, the increase for *D. simulans* falls off above 20° or below -20° latitude. From 0 to 20° the slope is significantly higher than from 20 to 45°. But these slopes are not significantly different from the single regression calculated for *D. melanogaster*.

As emphasised previously⁴ the adaptive significance of ovariole number and body weight must be explained by physiological traits such as reproductive capacity and resistance to environmental stress. Thus we decided to study another purely physiological trait, ethanol tolerance, the importance of which, in the ecology of the two species, has been discovered⁶. The variation of lethal concentration for adults as a function of

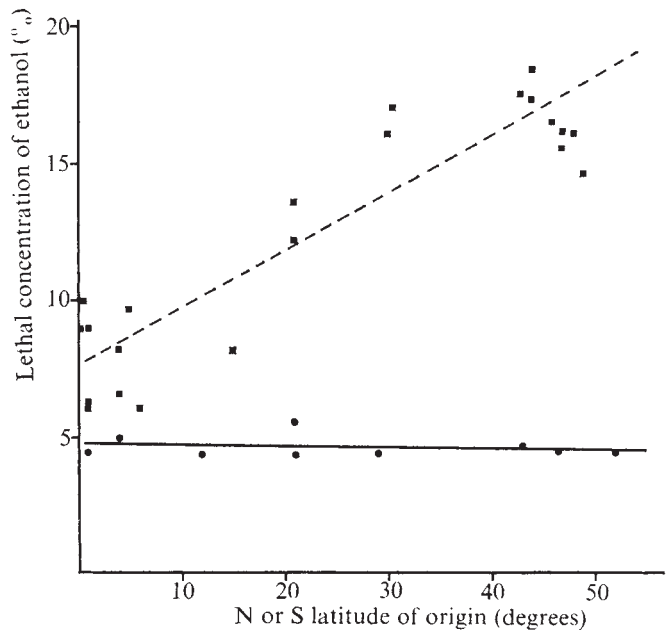


Fig. 2 Variation of ethanol tolerance in relation to the latitude of origin of the strains: ■, *D. melanogaster*; ●, *D. simulans*. Regression parameters (r , coefficient of correlation; b , slope; a , intercept, n , number of strains): *D. melanogaster*: $r = 0.90$, $b = 0.200 \pm 0.021$, $a = 7.69 \pm 0.63$, $n = 22$; *D. simulans*: $r = -0.23$, $b = -0.005 \pm 0.008$, $a = 4.79 \pm 0.25$, $n = 10$.

latitude of origin is shown in Fig. 2 (see ref. 7 for methods). In *D. melanogaster*, ethanol tolerance increases greatly with latitude; in *D. simulans*, however, the alcohol tolerance is lower and independent of latitude.

The higher ethanol resistance of *melanogaster* explains why it is the only species able to grow in highly alcoholic substrates such as fermented grapes in wine cellars^{6,8}. It seems probable that the geographical extension of *D. melanogaster* towards temperate countries occurred with a modification of its ecological niche, the species gradually becoming more linked to human activities such as artificial fruit fermentations. Much attention has been paid to the activity of alcohol dehydrogenase (Adh), which metabolises ethanol⁹. It now seems that alcohol tolerance is related to Adh activity (refs 8, 9, and B. Clarke, A. Elens and J. McDonald, personal communications). It is also known that, between the two widespread Adh alleles (slow and fast), fast usually shows a higher enzymatic activity¹⁰. Finally, a latitudinal cline (increase of fast frequency with latitude) has been demonstrated in the North American continent^{10,11}. All these data agree with the idea that the cline for alcohol tolerance described here corresponds, at a genetic level, to an increase of the fast allele frequency in temperate countries. Laboratory studies have demonstrated the selective value of the Adh alleles in experimental conditions^{8,12} and data from wild populations seem to demonstrate their selective values in nature.

The case of *D. simulans* is quite different, as the latitudinal adaptations seems to be independent of alcoholic food sources. This suggests that *D. simulans* may be less linked to (at least certain) human activities than *D. melanogaster*.

Latitudinal clines, on a shorter scale, have been demonstrated for biometrical polygenic traits in other (non-domestic) *Drosophila* species^{13,14}. The occurrence of similar data in two domestic species suggests that the natural selective mechanisms were the same—probably an adaptation to the average temperatures of the different regions.

An important problem is the time needed for the development of these genetic adaptations. Laboratory experiments indicate that less than 25 generations of artificial directional selection on equatorial strains are required to reach the higher weight, or higher ovariole number, of temperate strains. Moreover, experiments in population cages at different temperatures

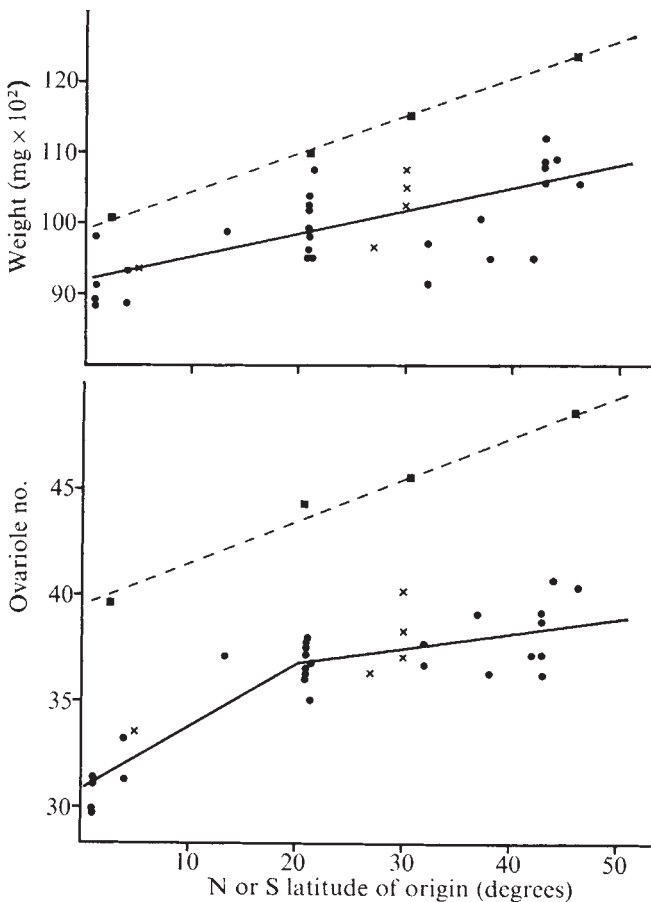


Fig. 1 Regression of female fresh weight and ovariole number on the latitude of origin of *D. melanogaster* and *D. simulans* strains. All measures were made on flies raised in standard laboratory conditions (25°C; killed yeast rearing medium; low larval density). For *melanogaster*, only the average points (■) of groups of strains are shown (see ref. 4). For *D. simulans*, each point corresponds to one strain: ×, American strains; ●, European or African strains. Regression parameters for *D. simulans* (r , coefficient of correlation; b , slope; n , number of strains): Female weight: $r = 0.69$, $b = 0.315 \pm 0.059$, $n = 32$; ovariole number, below 20°: $r = 0.91$, $b = 0.293 \pm 0.033$, $n = 17$; 20–50°: $r = 0.51$, $b = 0.080 \pm 0.028$, $n = 24$. In *D. melanogaster*, regression parameters for 51 strains: female weight: $r = 0.93$, $b = 0.503 \pm 0.029$; ovariole number: $r = 0.77$, $b = 0.179 \pm 0.021$.

have shown that biometrical characters of *D. pseudoobscura* can change significantly within a few years¹⁵. For *D. melanogaster* and *D. simulans*, the time needed for the genetic transformations induced by climatic changes is still a matter of debate. It may be that many thousands of generations were necessary for the fly populations to adapt from tropical to temperate conditions. Other species of the *melanogaster* subgroup and certain widespread domestic species such as *D. ananassae* have demonstrated an incapacity to extend their geographical distribution. We cannot, however, eliminate the commonly assumed possibility that the geographical extension of *D. melanogaster* and *D. simulans* is a recent event and that the colonisation of new regions was mediated by human transportations.

If this was indeed the case, a new question arises: why are so many tropical species, repeatedly introduced into temperate countries (for example, by escaping from laboratories) unable to found colonies? An answer may be found in the peculiarities of the genome of the cosmopolitan species. The study of allozymes, however, has failed to show systematic differences in level of polymorphism between cosmopolitan and other wild species¹⁶. Important differences exist in the chromosomal polymorphism of *D. melanogaster* and *D. simulans*: the former is highly polymorphic all over the world whereas the latter is monomorphic¹⁷. Such differences, however, seem to be of little importance for the adaptive capacities of the species.

Many problems thus remain in understanding the ecological genetics and the evolutionary capacities of the best known *Drosophila* species. A controversy has arisen over whether electrophoretic protein variants are "neutral" or "selective"¹⁸. Our data support a belief in the adaptive value of *Adh* alleles in nature. They also draw attention to the adaptive significance of biometrical polygenic systems which have often been neglected in current theories.

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Yamazaki and Maruyama^{8,9} concluded, from a survey of observed gene frequencies at 1,045 protein loci in a wide variety of species, that the data are compatible with the hypothesis of strict neutrality. A similar analysis¹⁰ of the data for three *Drosophila* species, however, has shown a significant excess of alleles with mean frequencies less than 0.1, by comparison with expectations based on the neutral infinite allele model of Crow and Kimura¹¹, presumably due to the accumulation of mutant alleles of minor deleterious effects¹².

In this report, a more extensive set of gene frequency data from electrophoretic surveys of species of *Drosophila* is analysed to assess the average magnitude of the selection pressures involved. The data are derived from 12 large scale studies of nine species of *Drosophila*¹³⁻²². The analysis involves only those enzymes for which at least three localities were sampled, and only those alleles reaching a frequency of 0.01 or more in at least one locality¹⁰.

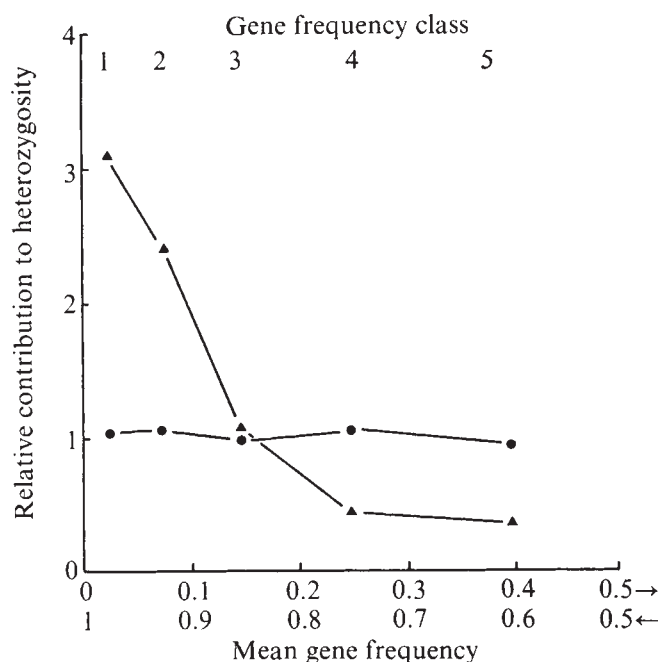


Fig. 1 The relative contribution of five gene frequency classes to heterozygosity for group I enzymes in *Drosophila* (▲), compared with expectations for the neutral charge class model (●) at equilibrium with $N\mu = 0.08$, determined by computer simulation.

Two types of genetic model have been used for comparison with the *Drosophila* data. The infinite allele model assumes that mutation to novel and individually distinguishable alleles occurs with frequency μ per generation. The charge class model, introduced by Ohta and Kimura²³, assumes that mutation occurs with frequency μ to novel alleles, of which a proportion α is not distinguishable electrophoretically from the parent allele, a proportion β gives rise to a protein differing by one unit of electric charge from the parental protein, and a proportion γ differs by two units of charge. Positive and negative changes in charge are assumed to be equally probable. Johnson²⁴ argued that the infinite allele and charge class models represent the two extremes of allelic non-identification when electrophoretic techniques are used.

The theoretical equilibrium gene frequency distribution in a random mating population of size N is known for the neutral infinite allele model²⁵, but not for the neutral charge class model. The equilibrium distribution for the charge class model has therefore been determined by computer simulation, using the values of $\alpha = 0.66$, $\beta = 0.32$, and $\gamma = 0.02$ calculated by Marshall and Brown²⁶ from the 'average protein' of King and Jukes²⁷, assuming that all mutations involve single random DNA base substitutions. The computer simulation was carried out with a population size of $N = 500$, allowing a period of

Influence of selection pressures on enzyme polymorphisms in *Drosophila*

KIMURA^{1,2} suggested that the widespread enzyme polymorphisms revealed by electrophoresis in *Drosophila*, man and other outbreeding species are due to the selective neutrality, or near-neutrality, of most mutations. Others have argued that the observed genetic variation is adaptive, since electrophoretic variants may differ in their biochemical properties³, different classes of enzymes show conspicuously different levels of heterozygosity⁴⁻⁶, and natural selection in the laboratory may lead to detectable gene frequency changes at enzyme loci⁷.

Table 1 Comparison of gene frequency distribution observed for group I enzymes in *Drosophila* and expected at equilibrium for genetic models involving slightly disadvantageous mutations ($Ns = 3$)

Genetic model	Relative contribution to heterozygosity of gene frequency class*					Mean heterozygosity
	1	2	3	4	5	
Infinite allele $N\mu = 0.14$	3.29 (± 0.11)	2.46 (± 0.06)	1.32 (± 0.04)	0.48 (± 0.04)	0.16 (± 0.05)	0.090 (± 0.002)
Charge class $N\mu = 0.40$	2.85 (± 0.14)	2.55 (± 0.09)	1.40 (± 0.04)	0.54 (± 0.03)	0.18 (± 0.04)	0.089 (± 0.003)
Group I enzymes	3.10 (± 0.45)	2.40 (± 0.38)	1.07 (± 0.22)	0.45 (± 0.12)	0.37 (± 0.14)	0.102 (± 0.012)

*Corresponding to mean gene frequencies falling in the intervals (1) 0.001–0.050 or 0.950–0.999; (2) 0.051–0.100 or 0.901–0.950; (3) 0.101–0.200 or 0.801–0.900; (4) 0.201–0.300 or 0.701–0.800; and (5) 0.301–0.700.
s.e. of estimation is given in parentheses.

20N generations for the attainment of a stationary gene frequency distribution. Thereafter, the gene frequencies in every generation for all electrophoretically distinguishable variants were tabulated over a period of 200N generations. The standard errors associated with the frequency distribution and mean level of heterozygosity were estimated from the observed variance among independent computer runs.

Figure 1 shows the gene frequency distribution for group I enzymes, that is, those which take part in glycolysis, the citric acid cycle or hexose monophosphate shunt, or whose substrates are in one of these pathways²⁸, compared with that for neutral alleles using the charge class model with the same overall level of heterozygosity. The ordinate in each case is the relative contribution of each gene frequency class to the total frequency of heterozygotes^{8,10}. For each of the 12 *Drosophila* surveys the contribution of a gene frequency class to heterozygosity has been expressed as a proportion of the total frequency of heterozygotes, and the relative contributions averaged over species with weights proportional to the number of independent alleles involved¹⁰.

The departure of the *Drosophila* group I enzyme data from expectations based on the neutral charge class model is statistically highly significant: the same applies to the neutral infinite allele model²⁹. There is a marked excess of alleles with mean gene frequencies in the range 0.0–0.1 or 0.9–1.0, and a corresponding deficiency in the range 0.2–0.8 (Fig. 1).

To simulate the *Drosophila* group I enzyme data, it is only necessary to suppose that all mutations are very slightly disadvantageous. Table 1 shows the results of simulation of an infinite allele model and a charge class model in which each mutant has a selective disadvantage s as a heterozygote, compared with the homozygote for the allele from which it arose, with all genotypic fitness values being determined additively. This model has been analysed deterministically by King and Ohta³⁰. In the charge class model, relative electrophoretic mobility is determined by differences in electric charge alone, but reproductive fitness does not depend on the net charge of the protein molecule. A charge class may contain two or more alleles with different selective values. Table 1 shows that selective pressures corresponding to $Ns = 3$ are sufficient to account for the observed departure of the group I enzyme data from the neutral hypothesis.

The gene frequency distribution for group II enzymes, that is, those not involved in glucose metabolism, is shown in Fig. 2. The corresponding neutral charge class model with an equivalent mean level of heterozygosity is given for comparison. The excess of alleles at extreme frequencies is not as marked as for group I enzymes, but it is, nevertheless, statistically highly significant: the same is true of the neutral infinite allele model²⁹.

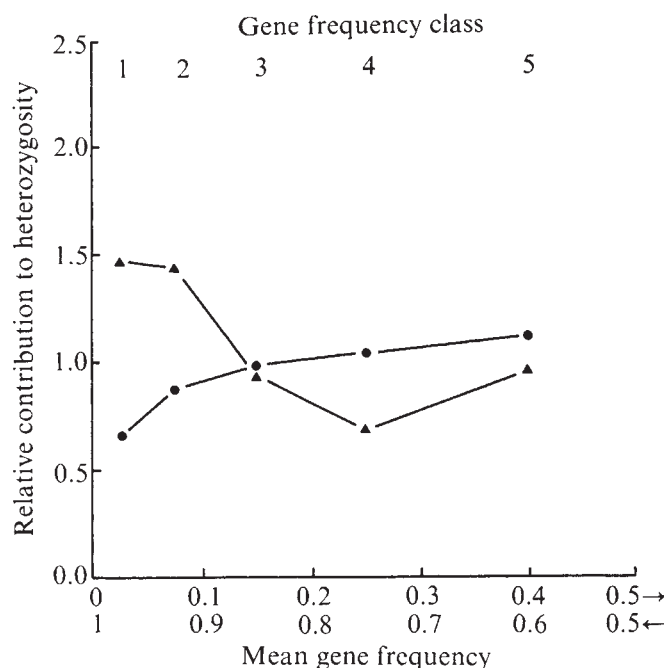


Fig. 2 *Drosophila* group II enzyme loci ($\blacktriangle$) compared with expectations for the neutral charge class model ($\bullet$) with $N\mu = 0.25$.

Table 2 shows that the models of mutation to slightly deleterious alleles with additive genotypic fitness values give equilibrium gene frequency distributions similar to that of group II enzyme loci when $Ns = 1$. The fit is not completely satisfactory, however, the data departing significantly from the model in

Table 2 Comparison of the gene frequency distribution observed for group II enzymes in *Drosophila* and expected at equilibrium for genetic models involving slightly disadvantageous mutations ($Ns = 1$)

Genetic model	Relative contribution to heterozygosity of gene frequency class					Mean heterozygosity
	1	2	3	4	5	
Infinite allele $N\mu = 0.14$	1.45 (± 0.09)	1.45 (± 0.08)	1.24 (± 0.04)	0.93 (± 0.02)	0.69* (± 0.06)	0.208 (± 0.012)
Charge class $N\mu = 0.40$	1.15 (± 0.08)	1.36 (± 0.08)	1.26 (± 0.04)	0.98† (± 0.02)	0.75† (± 0.05)	0.190 (± 0.010)
Group II enzymes	1.47 (± 0.17)	1.44 (± 0.10)	0.94 (± 0.14)	0.68 (± 0.12)	0.96 (± 0.08)	0.226 (± 0.015)

*Significant departure from group II enzyme data at $P = 0.01$.

†Significant departure from group II enzyme data at $P = 0.05$.
s.e. given in parentheses.

having an excess of allele frequencies in class 5, that is, in the range 0.3–0.7. Such an excess could be accounted for by two-allele polymorphisms at 5–10% of group II enzyme loci, maintained by balancing selection of sufficient intensity to keep gene frequencies in the range 0.3–0.7, with the remaining loci producing only deleterious mutants with N_s values of 2 or less²⁹. Among the group II enzymes, adenylate kinase, aldehyde oxidase and xanthine dehydrogenase contribute most to the excess of gene frequencies in the range 0.3–0.7.

It may therefore be concluded that the gene frequency data for group I and group II enzymes in *Drosophila* can be accounted for by mutation-selection balance at more than 95% of the loci concerned; and that the mean selection intensity influencing the deleterious mutant alleles at these loci is of an extremely low order, corresponding to N_s values in the range 1–3. Even if it were supposed that effective population size in species of *Drosophila* is as low as 1,000, the selective disadvantage of mutant heterozygotes implied by this analysis is of the order of 0.1–0.3%. Selection pressures of this magnitude are far too small to be detected experimentally³¹, and can only be revealed by extensive surveys of large numbers of enzyme loci.

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Evidence for a malaria mitogen in human malaria

HYPERGAMMAGLOBULINAEMIA is a prominent feature of human malaria. Serum levels of IgM and IgG rise rapidly in acute infections¹, and malaria has been implicated as an important cause of the raised levels of these immunoglobulins and the increased prevalence of rheumatoid factor and other autoantibodies found in healthy subjects in many parts of the tropics. Only a small proportion of the immunoglobulin produced in malaria can be shown to be specific antibody². The mechanisms underlying the production of large amounts of nonspecific immunoglobulin in malaria have not been established. Excessive immunoglobulin production could result from a breakdown in normal control mechanisms, perhaps as a consequence of the deletion of suppressor T cells. Alternatively, it could be due to the production during malarial

infection of a substance capable of stimulating lymphocytes nonspecifically³. Here we present evidence for the production of a mitogen in children with acute *Plasmodium falciparum* malaria.

Sera were obtained from 19 children with acute *P. falciparum* malaria at the time of presentation at hospital and from 16 healthy European adults taking regular malaria prophylactics. Blood for parasite culture was obtained from seven other children with acute *P. falciparum* malaria. None of these children had cerebral malaria or were clinically anaemic. Parasitaemia ranged from 1% to 15% parasitised erythrocytes with a mean of 7%. Blood for control cultures was obtained from six healthy Europeans who had never had malaria and who were taking regular malaria prophylactics. Heparinised blood, diluted 1.3 in RPMI 1640 medium, was passed through a column of CF 11 cellulose powder to remove white cells⁴. The diluted blood was washed through the column with RPMI 1640 and 5-ml samples collected. The first tube usually showed some haemolysis and was discarded. The next five or six tubes, containing only an occasional white cell, were collected and pooled. The red cells obtained were washed twice in fresh medium and a culture mixture was prepared containing 2 volumes of packed red cells, 2 volumes of inactivated foetal calf serum (FCS) or horse serum and 6 volumes of RPMI 1640. Culture was carried out in sterile conditions at 37 °C in 50-ml conical flasks loosely stoppered with cotton wool, in an atmosphere of 5% CO₂ in air.

After 24 h the culture was centrifuged and the supernatant retained. The remaining erythrocytes were washed twice in fresh medium, diluted 1.3 in RPMI 1640, layered over a Ficoll-Hypaque gradient and centrifuged at 400g for 30 min at 20 °C. This procedure removed remaining lymphocytes but resulted in the loss of some parasitised

Fig. 1 Mean stimulation ratio obtained on 3-d culture of lymphocytes from six immune Nigerians (●) and six non-immune Europeans (○) in the presence of different concentrations of supernatants obtained from culture of infected red cells. a, Supernatant 2; b, supernatant 3. Bars indicate the s.e.m.

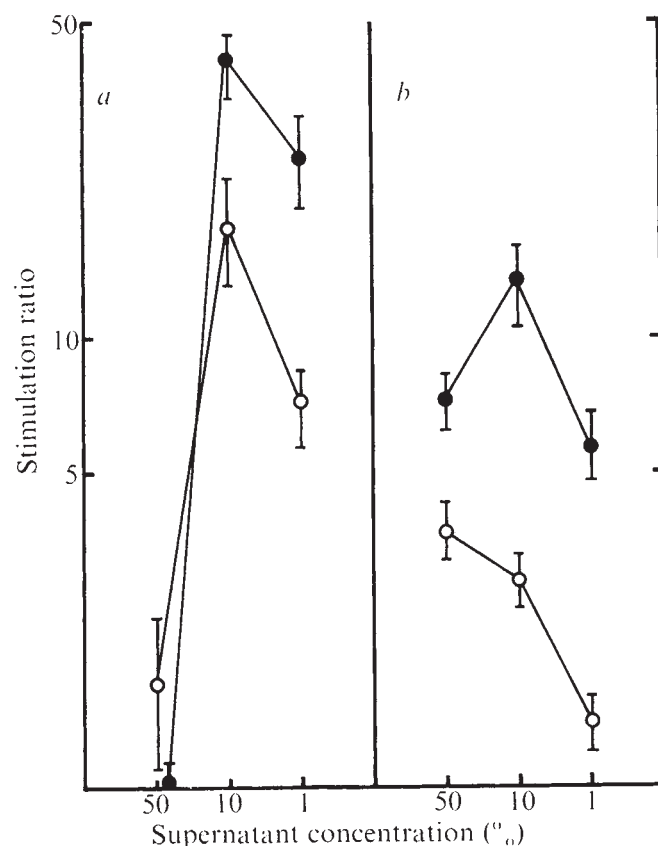


Table 1 Stimulation ratios

	50%	Supernatants 10%	1%	10%	Red cell lysates 1%	0.1%
Malaria cultures	2.0 (0.5-9.2)	4.2 (0.9-17.4)	2.0 (1.0-6.0)	2.4 (0.4-9.0)	1.9 (0.8-3.4)	1.5 (0.5-2.1)
Control cultures	0.9 (0.3-1.4)	0.9 (0.7-1.1)	1.0 (0.9-1.1)	1.0 (0.8-1.2)	1.2 (0.9-1.9)	0.7 (0.3-1.5)

Mean stimulation ratios were obtained on 3-d culture of lymphocytes from two non-immune subjects in the presence of different concentrations of supernatants and red cell lysates prepared from seven infected and six control erythrocyte cultures. Figures in parentheses indicate the range of stimulation observed.

erythrocytes and free malaria parasites. The sedimented erythrocytes were washed twice in medium and lysed with 0.87% ammonium chloride buffered with Tris-HCl buffer. After further washing the deposit was disrupted by sonication. The resulting supernatant, described here as a red cell lysate, was sterilised by Millipore filtration and stored at -40°C until tested. Protein concentrations of red cell lysate preparations were in the range of $1.3\text{--}5.5\text{ mg ml}^{-1}$. Blood from control subjects was treated in an identical way to blood from patients with malaria.

Sera, culture supernatants and red cell lysate preparations were tested for mitogenic activity on human peripheral blood lymphocytes and on mouse spleen cells. Lymphocytes were cultured in RPMI 1640 plus 10% FCS in microtitre trays at a concentration of 1×10^6 lymphocytes per ml for human peripheral blood cells and at a concentration of 3×10^6 lymphocytes per ml for mouse spleen cells. Eighteen hours before collection $1\text{ }\mu\text{Ci}$ of ^3H -thymidine was added to each well. Cells were collected on glass fibre disks with a Millipore manifold. Stimulation ratios have been calculated as the c.p.m. given by the test sample minus background divided by the c.p.m. given by the control sample minus background. Control cultures contained the same concentration of FCS or horse serum as the test samples. In each assay lymphocytes were tested against PHA at a final concentration of $50\text{ }\mu\text{g ml}^{-1}$.

Lymphocytes from a healthy European were cultured for 3 d in 10% serum obtained from 19 children with acute malaria and in 10% serum obtained from 16 controls. Three malaria sera, but none of the control sera, gave a stimulation ratio greater than 2 but other sera from the children with malaria were toxic, resulting in low cell viabilities and low stimulation ratios. Thus the mean stimulation ratio obtained on culture of lymphocytes in sera from children with acute malaria (1.0 ± 0.8) did not differ from the mean ratio obtained on culture in control sera (1.0 ± 0.5).

Supernatant and red cell lysate preparations obtained from culture of infected and control erythrocytes were tested for ability to stimulate lymphocytes obtained from two Europeans not immune to malaria (Table 1). Five out of seven supernatants and three out of seven red cell lysate preparations obtained from cultures of infected erythrocytes gave a stimulation ratio of 2 or more whereas none of six control supernatants or red cell lysate preparations gave stimulation of this degree. The mean stimulation ratio obtained with phytohaemagglutinin was 48.5 ± 19.6 .

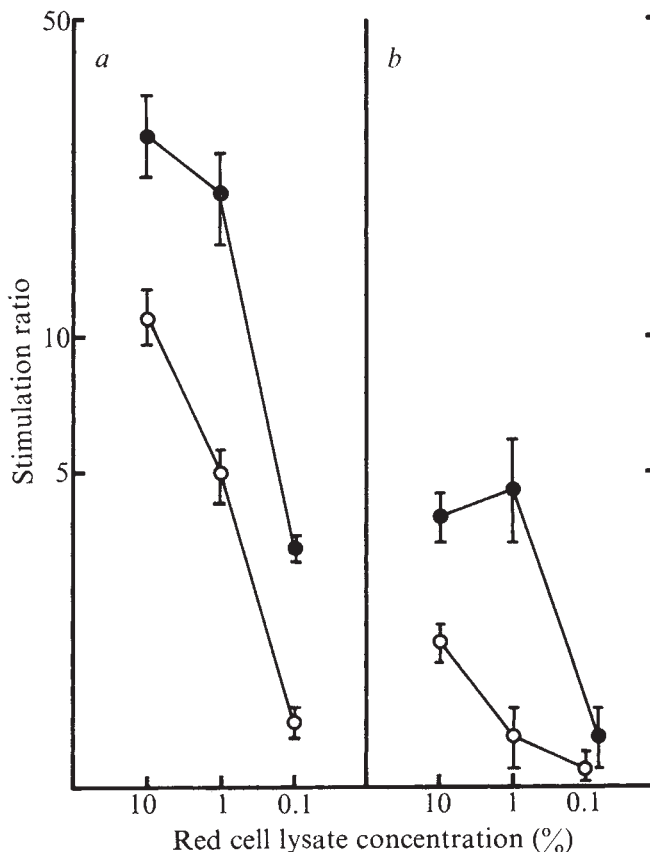
The two malaria supernatants and the two malaria red cell lysate preparations which gave the most marked stimulation in this initial experiment were tested for mitogenic activity on lymphocytes obtained from six adult Nigerians immune to malaria, on lymphocytes from six non-immune Europeans and on mouse spleen cells. Results for the two supernatants are shown in Fig. 1 and for the two red cell lysate preparations in Fig. 2. Although a wide range of responses was observed between different subjects each preparation gave a greater mean response in immune Nigerians than in non-immune Europeans. The different shapes of the dose-response curves obtained with supernatant 3 and red cell lysate 7 in immune and non-immune subjects also suggests increased sensitivity of lymphocytes

from immune Nigerians to these preparations. Lymphocytes from each subject responded to PHA with a mean stimulation ratio of 35.3 ± 19.7 for the European donors and a mean stimulation ratio of 12.3 ± 8.3 for the Nigerian donors. The low ratio obtained with lymphocytes from Nigerian donors reflects the high rate of isotope incorporation shown by their unstimulated cultures, a common finding in the local community. Mouse spleen cells showed a similar response to the two malaria supernatants and red cell lysate preparations as blood lymphocytes from non-immune Europeans.

A time-response curve was determined for supernatant 2 using lymphocytes from an immune and a non-immune donor. In each case maximal stimulation was observed around the third or fourth day of culture.

This study has shown that preparations obtained from short term culture of malaria-infected erythrocytes can stimulate lymphocytes from both immune and non-immune individuals. Leukocytes were removed from the culture preparations by an initial filtration through cellulose and by a subsequent Ficoll-Hypaque purification but the possibility that the stimulation observed in our experiments

Fig. 2 Mean stimulation ratios obtained on 3-d culture of lymphocytes from six immune Nigerians (●) and six non-immune Europeans (○) in the presence of different concentrations of red cell lysate preparations obtained from infected red cells. a, Lysate 2; b, lysate 7. Bars indicate the s.e.m.



was due to a mixed lymphocyte reaction, or even to a reaction to a red cell antigen, cannot be completely excluded. We believe this to be unlikely, however, for the following reasons. First, none of the control supernatants or red cell lysate preparations produced significant stimulation. Second, supernatants, presumably containing products released by parasite-lysed erythrocytes, produced more stimulation than cell-lysate preparations. The reverse situation would have been expected if a white cell or red cell antigen was involved. Finally, lymphocytes from those immune to malaria responded better than lymphocytes from non-immune subjects suggesting that the stimulatory factor was related to the presence of malarial products in the test preparations. It thus seems probable that the mitogenic factor demonstrated in our experiments was produced by malaria parasites or by parasitised red cells during the culture *in vitro*. Lymphocytes from malaria-immune Nigerians showed a greater response to the malaria culture preparations than lymphocytes from non-immunes, suggesting that these preparations contained both a malaria antigen capable of eliciting a specific cellular immune response by sensitised lymphocytes and a powerful nonspecific mitogen able to stimulate non-sensitised human peripheral blood lymphocytes and mouse spleen cells.

Our results thus agree with the finding that an antigen prepared by lysis of whole blood from monkeys infected with *P. falciparum* stimulated markedly lymphocytes from most subjects who had experienced malaria, and that at high antigen concentrations, lymphocytes from non-immune controls and even lymphocytes from cord blood responded³. These findings suggest this malaria preparation also contained both an antigen able to elicit a specific cell-mediated immune response to malaria and a nonspecific mitogen.

There is increasing evidence of the development of specific cell-mediated immunity during human malaria infection but it has yet to be established whether this has any protective role. It has been suggested that sensitised T lymphocytes function as helper cells, aiding the rapid production of antibody to newly arising antigenic variants⁶. Macrophage activation as a result of lymphokine production could also have a protective role by enhancing phagocytosis of parasitised erythrocytes.

The results of this study support the view that a mitogenic factor is produced during the course of human malaria infection but it has yet to be established whether it plays any part in the production of the hypergammaglobulinaemia characteristic of the infection. We are currently investigating whether it acts predominantly on B lymphocytes or T lymphocytes and whether it can stimulate immunoglobulin synthesis by lymphocytes *in vitro*.

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As OFAs also seem to be shed from cell surfaces into blood and other body fluids, there is particular interest in the possible diagnostic value of detection of OFAs such as carcinoembryonic antigen^{1,2} and α foetoprotein³. In spite of many studies in this latter area, there is little information on either the basic function of OFA components or of their possible role in immune responses to tumours *in vivo*. Their frequent association with rapidly dividing cell populations suggests a role related to growth and differentiation rather than in the neoplastic process itself. Embryonic exposure of the normal individual to OFAs may induce immunological tolerance to these antigens, thus effectively negating any subsequent potential role of OFAs to act as tumour-associated transplantation antigens capable of eliciting an anti-tumour response *in vivo* in the host².

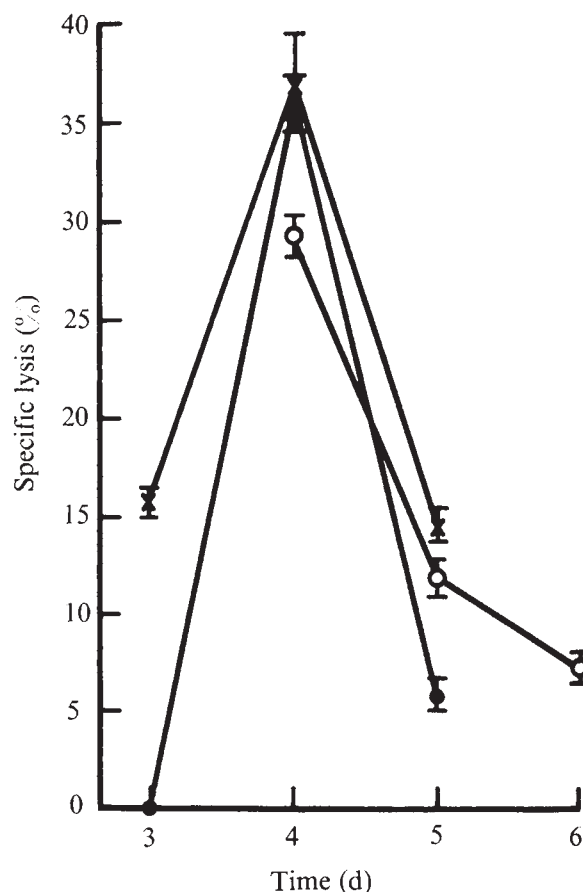


Fig. 1 Specific lysis of ⁵¹Cr-labelled target tumour cells by cytotoxic lymphocytes activated *in vitro* by syngeneic 14-d foetal liver cells, at a responder-stimulator ratio of 10:1. Cytotoxic lymphocytes were collected at indicated times (d) after initiation of culture, then held for 24 h in fresh medium before assay with ⁵¹Cr labelled MPC-11 cells (●, ○) or Wehi-164 cells (x). (CL-target ratio 100:1). Error bars are standard errors of triplicates.

For detailed investigations of this situation, animal models of OFAs are particularly appropriate, and various reports of OFAs on animal tumours have appeared^{2,4-6}. These include inhibition of chemical or viral carcinogenesis or suppression of tumour growth by foetal tissue immunisation, and in the reverse direction, inhibition of foetal cell proliferation by immunisation with tumour cells⁴⁻⁷. Analysis of the cellular mechanisms of immune responses to OFA in these situations has been limited, with one report demonstrating the presence of cytotoxic lymphocytes in the spleens of mice immunised with foetal cells, capable of lysing ⁵¹Cr-labelled tumour cells⁸. Analysis of the cellular components involved in anti-tumour responses has been aided by the development of totally *in vitro* systems for the induction and assay of anti-tumour immunity⁹⁻¹¹, making possible the control of variables which are difficult to evaluate *in vivo*. We now describe the *in vitro*

Lymphocyte activation *in vitro* to murine onco-foetal antigens

ONCO-FOETAL antigens (OFAs) are operationally defined as immunogenic cell surface components present on various types of malignant and foetal cells, but not present on adult cells^{1,2}.

generation of cytotoxic lymphocytes by syngeneic foetal liver cells, and their ability to lyse syngeneic tumour cells.

The choice of foetal liver^{6,7} and the tumours used in this study was based on the results of investigations *in vivo*, demonstrating the ability of each tissue to provoke immune responses against the other (S.C., Wallis, and N.L.W., unpublished). The *in vitro* techniques used are described in detail elsewhere¹¹, and were briefly as follows. In all cases the microculture system of 4-ml compartments containing 15×10^6 responder cells was used. Irradiated (5,000 rad) BALB/c 14-d gestation foetal liver cells (stimulator cells) were cultured with 7-week-old male BALB/c spleen cells (responder cells) in Eagle's MEM containing non-essential amino acids, 5% foetal calf serum (FCS), and 10^{-4} M 2-mercaptoethanol at 37 °C in 10% CO₂ in air, using various ratios of stimulator-responder cells. At various times after initiation of culture, cells were collected and resuspended in Dulbecco's modified Eagle's medium (DMEF) supplemented with 10% FCS for a further 24 h before assay. On the day of assay, tumour target cells were labelled with ⁵¹Cr in DMEF and mixed with the cultured lymphocytes at a cytotoxic lymphocyte (CL) to target (T) ratio of 100:1 and 50:1. After incubation at 37 °C for 4 h and 45 °C for 1 h, the amount of ⁵¹Cr released to the supernatant was measured. Background (BG) lysis was determined by measuring the amount of ⁵¹Cr released without CL, and the total amount of releasable ⁵¹Cr (maximal count) (MC) was determined by adding Zaponin (improved lysing agent for white blood cell counts). The percentage specific lysis is [(test count - BG)/(MC - BG)] × 100. The tumour lines used were a methylcholanthrene-induced BALB/c fibrosarcoma¹² WEHI-164, and a BALB/c plasmacytoma, MPC-11, (provided by Dr M. Scharff, Albert Einstein College of Medicine, New York). In several studies, inhibition of specific lysis was attempted by adding unlabelled foetal liver, adult spleen or tumour cells at various ratios to the ⁵¹Cr target tumour cells during the assay phase.

The generation of CL induced by syngeneic foetal liver cells and capable of lysing syngeneic tumour cells is demonstrated in Fig. 1. Culture of cells for 4 d before the preincubation and lysis assay resulted in optimal specific lysis of either tumour. In comparison the percentage specific lysis of tumour targets using BALB/c lymphocytes cultured with irradiated adult BALB/c spleen cells averaged $9 \pm 2\%$, regardless of assay day. Although there was some variation in the degree of lysis of the target cells from one experiment to another there was always at least a threefold increase of ⁵¹Cr release with BALB/c anti-foetal liver compared with anti-adult spleen. As other studies^{13,14} have inferred the *in vitro* generation of self-reactive CL, it is essential to make this comparison of adult with foetal stimulator cells in these studies. Our data on specific lysis with anti-self-reactive cells and the inhibition data below, do not suggest that the reactivity observed against foetal liver and tumour cells is of this auto-reactive nature.

Varying the ratio of responder to stimulator cells revealed optimal lysis at 10:1 with both tumour targets, whereas in studies with specific anti-tumour immune responses, optimal ratios were 100:1 for MPC-11 (ref. 11), and 1,000:1 for WEHI-164 (R.B. and N.L.W., unpublished), again stressing the different type of antigenic system involved in the OFA study. Preliminary studies have also indicated that the gestational age of foetal liver effects the level of specific lysis generated, with a progressive decrease from 13 to 16 d of foetal age.

Evidence for specificity of cytotoxic lymphocyte activation comes from blocking experiments using non-labelled cells. Specific lysis of ⁵¹Cr target tumour cells by cytotoxic lymphocytes could be inhibited quantitatively by addition of foetal liver cells or Wehi-164 cells during the assay (Fig. 2), whereas there was little effect with adult spleen cells. In such experiments we consistently observed marked inhibition with Wehi-164 cells, but considerably less with MPC-11 cells, possibly indicating quantitative differences in the expression of OFA on different tumours. The degree of inhibition with foetal liver cells has been at least three times greater than that observed with adult

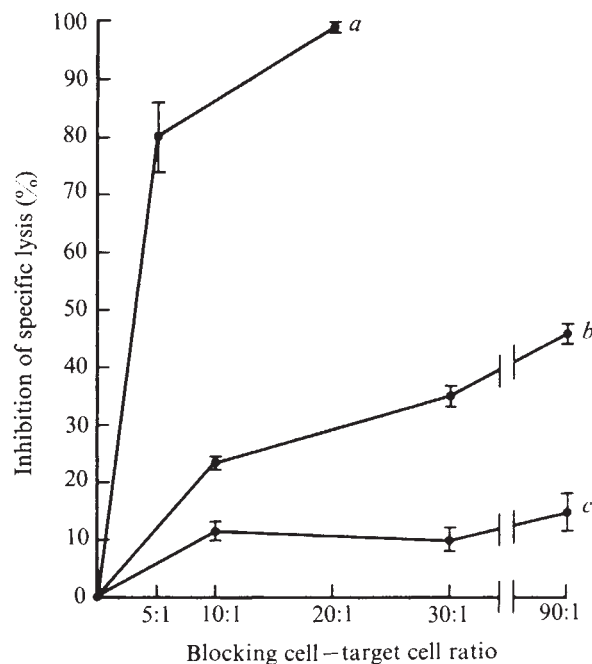


Fig. 2 Inhibition of ⁵¹Cr release from labelled Wehi-164 cells (CL-target ratio 50:1) by unlabelled Wehi-164 (a), 14-d foetal liver (b) and adult spleen cells (c) at various ratios of blocking cells to labelled target cells. The error bars represent standard errors of triplicate assays.

spleen cells, particularly at high blocking ratios. Whether the requirement for a relatively large number of foetal liver cells as either stimulators or blockers of CL reflects the expression of OFA on only subpopulations of foetal liver cells (for example, haematopoietic stem cells^{6,7}) or low amounts on most cells, remains to be elucidated. Preliminary studies have suggested the presence of OFA in foetal tissues other than liver. When degrees of blocking obtained with foetal or tumour cells are compared, it should be noted that the tumour cells used are of considerably larger volume.

The data presented here are consistent with the thesis that murine foetal cells have antigens in common with several murine tumour cells, which are not present on adult normal cells. The ability to generate *in vitro* cytotoxic lymphocytes to these antigens now permits further analysis of the tumour-host relationship involving OFA. As the two tumours used in this study do not apparently share *in vivo* tumour-associated transplantation antigens, and yet do share OFA, we are now determining whether the *in vitro* expression of CL represents T cell or B cell-mediated responses, perhaps released *in vitro* from a persistent *in vivo* suppression mechanism, as proposed for the *in vitro* generation of autoreactivity¹³.

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Altered serological and cellular reactivity to H-2 antigens after target cell infection with vaccinia virus

Mice generate cytotoxic T lymphocytes (CTL) which are able to lyse virus infected target cells *in vitro* after infection with lymphocytic choriomeningitis virus (LCMV) and pox-viruses¹⁻³. CTL kill syngeneic and semiallogenic infected cells but not allogenic infected targets. Target cell lysis in these systems seems to be restricted by H-2 antigens, especially by the K or D end of the major histocompatibility complex (MHC). In experiments where virus specific sensitised lymphocytes kill virus infected allogenic target cells⁴ the effector lymphocytes have not been characterised exactly. Recent investigations suggest that the active cell in this assay, at least in the measles infection, is a non-thymus derived cell (H. Kretz, personal communication). An H-2 restriction of cell mediated cytotoxicity (CMC) to trinitrophenol (TNP)-modified lymphocytes has also been described⁵. Zinkernagel and Doherty⁶ postulated that the CTL is directed against syngeneic H-2 antigens and viral antigens and they suggested an alteration of H-2 induced by the LCMV infection. Earlier⁷ we found a close topological relationship between H-2 antigens and the target antigen(s) responsible for CMC in the vaccinia system. Here we report experiments which were carried out to prove alteration of H-2 after infection of L-929 fibroblasts with vaccinia virus.

Alteration of H-2 antigens was studied by immunofluorescence. L-929 cells after 1 h infection with 10 TCID₅₀ per cell vaccinia virus strain WR and 19 h incubation in serum-free medium, as well as normal L-929 cells were stained by indirect immunofluorescence. L cells (5×10^6) were incubated in 100 λ H-2^k (charge D 3b, raised in recipient-donor strains (C3H-H2^d $\times$ 129) anti-C3H; genotypes ($K^d D^k \times K^b D^b$) anti- $K^k D^k$. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52 and haemagglutinating for specificity 3, (ref. 8) for 30 min, 37 °C and 4 °C and after washing 3 times in PBS incubated in 100 λ FITC-labelled rabbit anti-mouse IgG in the same conditions. After the cells were washed three times, viability was tested (> 90%) and slides were prepared without previous fixation of cells. All of the normal fibroblasts had a dense and regular fluorescence, whereas about 25% of the infected cells lacked H-2 fluorescence. The remaining 75% cells had an irregular and more coarse-grained staining.

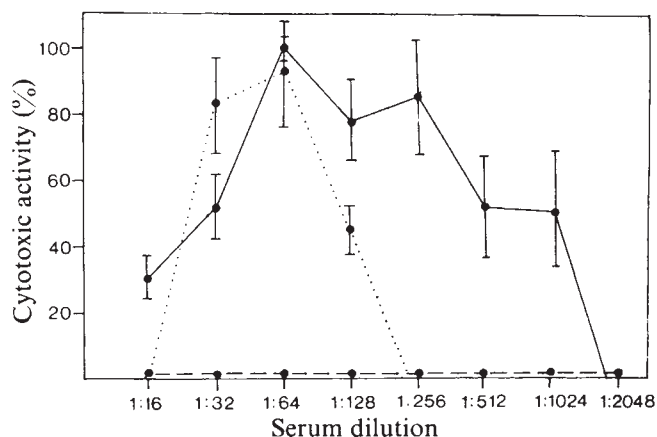


Fig. 1 H-2^k alloantiserum absorption with normal and vaccinia infected L-929 cells. ●—●, cytotoxic activity of anti H-2^k serum (⁵¹Cr release) against C3H spleen lymphocytes. ●.....●, cytotoxic activity of anti H-2^k serum after 30 min absorption at 37 °C with 1×10^8 vaccinia virus infected L cells (20 h infected 10 TCID₅₀ per cell vaccinia strain WR). ●-----●, cytotoxic activity of anti H-2^k serum after absorption with 1×10^8 normal L-929 cells: Serum dilutions were performed in Eagle's MEM. Each point represents $N = 4$ values $\pm$ s.d. Differences are statistically significant ($P < 0.001$). Maximal ⁵¹Cr release by H-2^k alloantiserum before absorption was calculated as 100% cytotoxic activity (Antibody mediated ⁵¹Cr release ranged from 40-65% of total incorporated chromium). Controls included spontaneous lysis of targets alone, lysis in presence of complement alone, H-2 antibodies alone, normal mouse serum alone and normal mouse serum together with complement.

Absorption studies were performed to quantify the possible H-2 alteration seen in immunofluorescence. Different numbers of viable infected and non infected L-cells (2.5×10^7 – 1×10^8) were incubated with 0.5 ml anti-H-2^k serum for 30 min at 37 °C. After this, the cytotoxic titre of the absorbed serum was tested in the ⁵¹Cr-release assay using C3H spleen lymphocytes (H-2^k) as targets and guinea pig serum as complement source (Figs 1 and 2). Then the lysis of target cells was assayed by Trypan blue exclusion using rabbit complement. Both tests gave nearly the same results.

Serum D-3b was absorbed quantitatively five times, with comparable results. There was a constant reduction of H-2 alloantiserum absorbing capacity of L-929 cells infected with vaccinia virus (Fig. 1). Using 1.0×10^8 normal cells for absorption we found no cytotoxicity in the ⁵¹Cr-release assay. The complete absorption was not simulated by anti-complementary activity of the H-2 serum since the absorbed serum showed no more H-2 fluorescence, and absorption with increasing amounts of normal L cells showed a stepwise decrease of cytolytic activity. Serum absorbed with the same amount of infected cells showed cytotoxic activity up to a serum dilution of 1:128. After absorption with 2.5×10^7 and 5×10^7 cells the anti-H-2^k serum showed 20-40% more cytotoxic activity when infected cells were used for absorption (Fig. 2).

Table 1 Blocking of CMC against target cells infected with vaccinia virus

Lymphocytes*	Target cell	Cr release†	Specific lysis‡
Normal DBA/2	L-929	20.1 $\pm$ 1.2	
Normal C3H	not infected	18.5 $\pm$ 0.7	
DBA/2 sensitised to H-2 ^k		64.4 $\pm$ 0.8	44.3§
C3H sensitised to vaccinia virus		19.4 $\pm$ 1.3	
Normal DBA/2	L-929	21.2 $\pm$ 0.9	
Normal C3H	vaccinia virus infected	23.4 $\pm$ 0.8	
DBA/2 sensitised to H-2 ^k		22.5 $\pm$ 0.7	
C3H sensitised to vaccinia virus		72.8 $\pm$ 1.4	49.4§

*Lymphocytes from a pool of six mice per group. Virus sensitisation was performed by intraperitoneal injection of 1 ml 1×10^6 TCID₅₀ ml⁻¹ vaccinia virus six days previously. Sensitisation to H-2^k was achieved by intraperitoneal injection of 1×10^8 C3H spleen lymphocytes 10 d previously. Killer-target cell ratio, 100:1.

†Mean of percentage ⁵¹Cr release $\pm$ s.e.m. of 5 wells per group.

‡Specific lysis (%) = (⁵¹Cr release (%) by sensitised lymphocytes) – (⁵¹Cr release (%) by normal lymphocytes).

§Differences are statistically significant ($P < 0.001$).

The influence of target-cell infection on CMC was tested. Since the effector phase of the allograft reaction, which is reflected *in vitro* by the CMC of allogenic cells, is directed mainly against serologically defined (SD) antigens¹⁰, modifications of these antigens should influence the degree of CMC. DBA/2 mice (genotype H-2^d) were sensitised to H-2^k by intraperitoneal injection of 1×10^8 spleen cells from C3H mice. Ten days later, the DBA/2 lymphocytes were collected and the cytolytic activity of the CTL tested against infected and non-infected L-929 target cells in a ⁵¹Cr-release assay³. Table 1 shows the results of an experiment representative of the four carried out. After incubation of the CTL sensitised to H-2^k with the normal L cells we found a specific release of 44.3% of the incorporated chromium. There was, however, no specific lysis of the allogenic target cells infected corresponding to the serum absorption assays with vaccinia virus. In the immunofluorescence control the target cells had vaccinia virus surface antigen fluorescence and altered H-2 fluorescence as described above. In spite of serologically demonstrable H-2 antigens, the CTL could not kill the infected allogenic cells. With syngeneic attacker cells sensitised to vaccinia virus we observed significant specific lysis of the infected target cells.

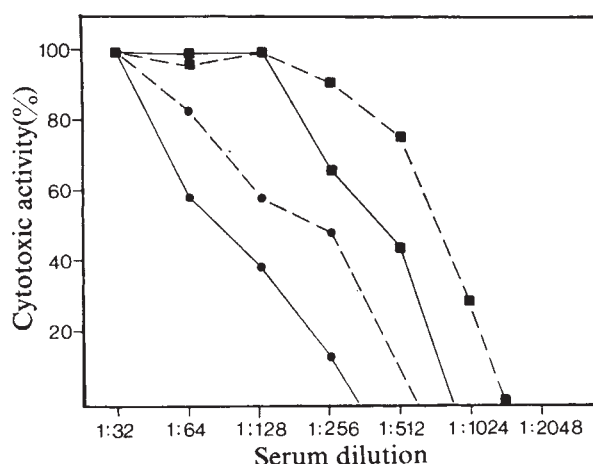


Fig. 2 Comparison of quantitative absorption by different numbers of vaccinia virus infected and normal L-929 cells. ●—●, H-2^k (0.5 ml) absorbed on 0.5×10^8 L cells; ●—●, 0.5×10^8 vaccinia virus infected L cells; ○—○, 0.25×10^8 L cells; ■—■, 0.25×10^8 vaccinia virus infected L cells. Maximal ⁵¹Cr release by H-2^k antiserum before absorption was calculated as 100% cytotoxic activity.

These data indicate that infection of L-929 fibroblasts with vaccinia virus may result in a reduction and an alteration of H-2 antigenic sites. It has been shown by serum absorption tests that tumour viruses can increase or diminish the level of H-2 specificities^{11,12}.

H-2 antigen expression is cell cycle dependent¹³ and possibly the quantitative alteration after vaccinia virus infection could be caused by a virus-induced nonspecific blocking of the metabolism of membrane proteins. Using enteroviruses as infective agents we were not able to find a reduced H-2 allo-anti-serum absorbing capacity or an inhibition of lysis of allogeneically infected cells in the CMC. Expression of viral surface antigens seems, therefore, to be a prerequisite for antigenic alteration. Rearrangement of H-2 antigen specificities¹⁴ accompanied by virus-induced inhibition of membrane fluidity does not seem to be a sufficient explanation for the inhibition of T-cell effector function, since antigenic recognition is possible after fixation of cells¹⁵.

If the expression of H-2 in infected and non-infected cells were identical by serological means and infection induces only quantitative differences of H-2 antigen expression, the cells should be lysable in the CMC against alloantigens¹⁶. There is, however, not only slight reduction, but complete blocking of CMC. This could mean that there is a virus-induced qualitative

change of H-2 antigenicity or a steric hindrance of the target antigen for the T cell sensitised to alloantigen. Different localisation of serologically and cellularly detectable antigenic sites on the same molecule could also explain the different results of antibody binding and allogenic CMC. The latter possibility is supported by the finding that CMC between cells identical in SD antigens and different only in H-2 alloanti-serum absorbing capacity occurs¹⁷⁻¹⁹. Reduction of CMC against allogenic cells has been obtained by Gardner *et al.*²⁰ in the ectromelia system while modification of cells with TNP did not alter CMC to H-2 alloantigens⁵. These contradictory results may reflect the different sizes of the modifying agents or the different process and extent of alteration.

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Modulation of macrophage Fc receptor expression *in vitro* by insulin and cyclic nucleotides

THE capacity of certain cells to bind antibody or immune complexes through receptors at the cell surface may be important in both inductive and effector limbs of the immune response. Macrophages¹, B lymphocytes², activated T lymphocytes³ and certain tumour cells⁴ express such receptors which interact with the Fc portion of IgG (Fc receptors). I have shown that guinea pig macrophages are heterogeneous in their capacity to bind antibody, that the expression of Fc receptors is greatly increased during cell activation *in vivo* and *in vitro* and that this activation of receptor expression is inhibited by a factor(s) present in fresh autologous serum⁵. Here I show that changes in macrophage Fc receptor expression *in vitro* are modulated by insulin and by cyclic nucleotides.

Normal peritoneal cells were extracted aseptically from untreated outbred guinea pigs by washing the peritoneal cavity with 25 ml of RMPI 1640 tissue culture medium supplemented with 10% foetal calf serum (FCS) and containing heparin (5 U ml⁻¹). Samples (1 ml) of this suspension, which contained approximately 1×10^6 macrophages per ml, were placed immediately in each chamber of dual tissue culture slides (Lab-Tek Products). Cells from more than one animal were never pooled. These cells were incubated at

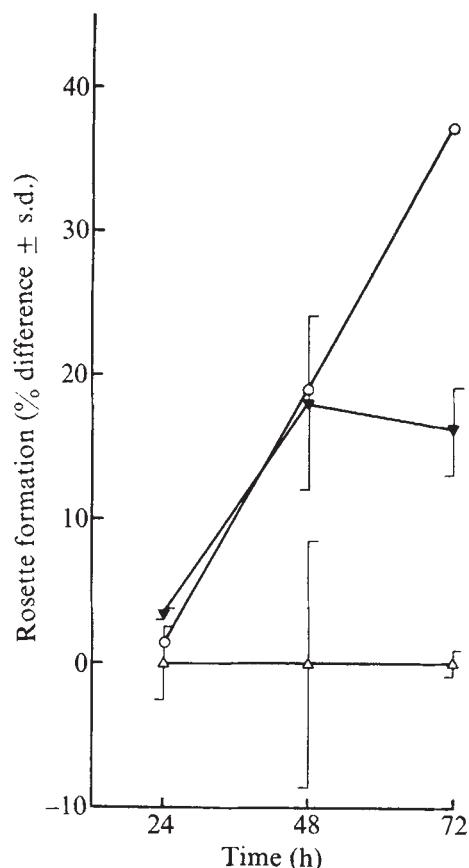


Fig. 1 The data are normalised by taking the percentage rosette formation in cultures exposed to insulin at $50 \mu\text{g ml}^{-1}$ (Δ) as a reference and plotting the difference between these values and values in control cultures ($\circ$) and cultures exposed to insulin at $10 \mu\text{g ml}^{-1}$ ($\blacktriangledown$). Each point represents the mean $\pm$ s.d. of three independent experiments.

37°C for 2.5 h after which non-adherent cells were removed by washing the monolayer twice with heparin-free medium. The resultant monolayers, which consisted of macrophages of $>95\%$ purity, were cultured in RPMI 1640 supplemented with 20% FCS in an atmosphere of 5% CO_2 in air at 37°C . In all cultures the medium was replaced every 24 h with a fresh preparation. Control cultures received RPMI with 20% FCS whereas experimental cultures received the same medium containing one of the following: insulin, dibutyryl cyclic AMP, dibutyryl cyclic GMP, 3-isobutyl-1-methylxanthine (IBMX), dibutyryl cyclic AMP+IBMX, dibutyryl cyclic GMP+IBMX, at the concentration specified.

Guinea pig IgG anti-sheep erythrocyte antibody was prepared from the sera of animals mounting a secondary response to sheep erythrocytes in Freund's complete adjuvant⁵. Antibody-sensitised sheep erythrocytes (EA) were prepared by incubating the cells with various concentrations of this antibody, and a degree of EA sensitisation appropriate for monitoring changes in macrophage Fc receptor activity was determined by obtaining a dose-response curve for rosette formation on monolayers at day 0 of culture, as described previously⁵. On subsequent days of culture the percentage rosette formation in control cultures in one chamber of a dual chamber slide was compared with that in experimental cultures in the other chamber. Thus, after rosette formation with a single preparation of EA, cultures were washed three times in Hanks' balanced salt solution, fixed with 1% glutaraldehyde and stained with buffered Giemsa. Five hundred cells were counted per culture and macrophages bearing five or more erythrocytes were scored as rosettes and expressed as a percentage of the total cells.

In control cultures the percentage rosette formation increased markedly as expected during 72 h of culture. When cells were cultured in medium containing $10 \mu\text{g ml}^{-1}$ or $50 \mu\text{g ml}^{-1}$ of insulin, however, the increase in receptor expression was inhibited to a degree related to the concentration of insulin present (Fig. 1). Existing Fc receptors were not blocked by insulin, but the increase in receptor expression was inhibited in a dose related manner. The effect therefore is unlikely to be due to steric hindrance of Fc receptors by surface-bound insulin. This *in vitro* effect resembles that of fresh autologous serum in that activation is inhibited⁵. Insulin did not significantly affect cell viability. When cells were cultured in the presence of dibutyryl cyclic AMP ($5 \times 10^{-5} \text{ M}$) the increase in receptor expression was again inhibited (Fig. 2). IBMX at the same concentration also inhibited the change in receptor expression. IBMX is a potent inhibitor of phosphodiesterases which hydrolyse cyclic AMP to form 5'-AMP (ref. 6); its presence should thereby cause an accumulation of intracellular cyclic AMP. When both dibutyryl cyclic AMP and IBMX were present the increase in receptor activity was inhibited to a greater degree than when either was present alone (Fig. 2). In the latter cultures Fc receptor activity remained at about the same level as that initially present. This synergistic effect would be expected to result from inhibition of the hydrolysis of both intracellular cyclic AMP

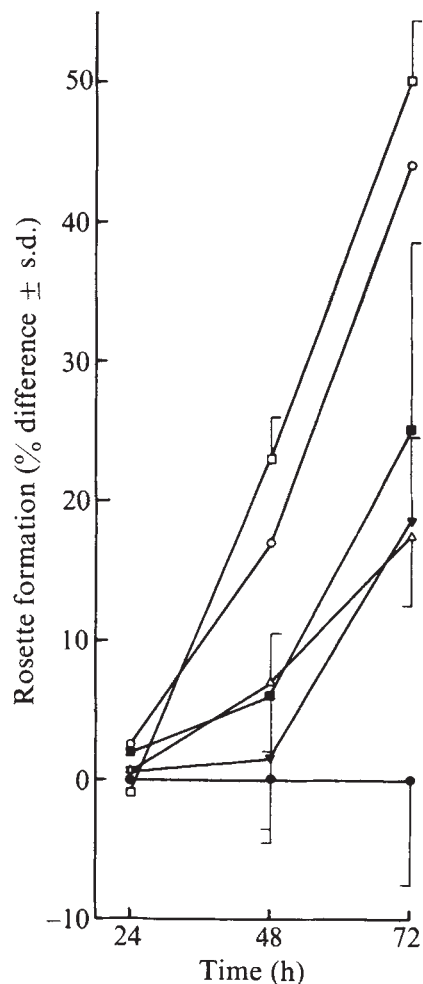


Fig. 2 The data are normalised by taking the percentage rosette formation in cultures exposed to $5 \times 10^{-5} \text{ M}$ dibutyryl cyclic AMP + $5 \times 10^{-5} \text{ M}$ IBMX ($\bullet$) as a reference and plotting the difference between these values, control values ($\circ$), and values in cultures exposed to the following: dibutyryl cyclic AMP (Δ), dibutyryl cyclic GMP ($\square$), IBMX ($\blacktriangledown$), dibutyryl cyclic GMP + IBMX ($\blacksquare$) all at a concentration of $5 \times 10^{-5} \text{ M}$. Each point represents the mean $\pm$ s.d. of four independent experiments. For clarity only one s.d. is shown on each point.

and dibutyryl cyclic AMP added to the medium. It further indicates that cyclic AMP, rather than 5'-AMP or other catabolites, exerts the observed effect. Dibutyryl cyclic MP (5×10^{-3} M) slightly augmented the increase in receptor expression (Fig. 2). When both dibutyryl cyclic GMP and IBMX were present at the same concentration the inhibitory effect of IBMX prevailed (Fig. 2). Presumably the effect of accumulated intracellular cyclic AMP is more telling than that of dibutyryl cyclic GMP added to the extracellular medium. Cell viability did not differ significantly between control and experimental cultures. Dibutyryl cyclic AMP at a concentration of 5×10^{-6} M had no effect on receptor expression (data not shown).

Cyclic nucleotides have been implicated in the control of various immunological and inflammatory functions⁷. In general cyclic AMP inhibits and cyclic GMP augments such functions as T-lymphocyte cytotoxicity⁸, lymphocyte proliferation and antibody production⁹, histamine release by mast cells¹⁰, lectin-induced uptake of calcium by T lymphocytes¹¹. My results are compatible with a model of macrophage activation in which the expression of Fc receptors is a function of antagonistic signals: insulin or hormones resembling insulin exerting a stabilising influence by maintaining a high intracellular cyclic AMP: cyclic GMP ratio, and other signals, possibly lymphokines, exerting an activating influence by lowering this ratio. The least requirement for activation *in vitro* may be the removal of the stabilising influence. It is interesting that a receptor for insulin has recently been demonstrated on human monocytes (R. H. Schwartz, A. R. Bianco, B. S. Handwerker, and C. R. Kahn, unpublished). A similar mechanism may also control T-lymphocyte Fc receptor expression which resembles that of macrophages⁵ in so far as the development of receptors during culture occurs in FCS but not in autologous serum¹². The expression of Fc receptors on cells derived from a murine myeloma has been shown to be inhibited by cholera exotoxin which increased intracellular cyclic AMP levels¹³.

An understanding of the mechanism controlling macrophage Fc receptor expression is important because changes in such expression may have a regulatory role both in the induction of an immune response and in the effector mechanisms of immunological surveillance. Increased receptor expression may facilitate the presentation of antigen to T lymphocytes since macrophage-bound antibody can mediate such presentation^{14,15}. It may also facilitate the killing of target cells by macrophages and I suggest that this explains the increased antibody dependent cytotoxicity exhibited by cultured macrophages¹⁶. Since circulating tumour antigen or antigen-antibody complexes seem to suppress the cellular response against tumours¹⁷, increased macrophage Fc receptor expression might also be expected to facilitate this response by accelerating the clearance of immune complexes.

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Molecular differences of exposed surface proteins on thrombasthenic platelet plasma membranes

THE platelet plasma membrane contains several proteins of different molecular weights. Three of these proteins stain for carbohydrate with periodic acid-Schiff reagent and have been termed glycoproteins I, II and III (molecular weights 150,000, 124,000 and 106,000, respectively)^{1,2}. It has been suggested that the bleeding tendency observed in Glanzmann's thrombasthenia results from abnormalities of the platelet membrane³⁻⁶. Nurden and Caen⁷ showed that glycoprotein II was absent in a crude membrane fraction isolated from thrombasthenic platelets. We have now investigated the surface composition of thrombasthenic membranes by the lactoperoxidase iodination technique² and found a low concentration of glycoprotein II, and other molecular differences.

When normal platelets are iodinated by the lactoperoxidase technique, glycoproteins I, II and III are labelled together with numerous other polypeptides of lower molecular weight². In addition a membrane component is labelled that has a molecular weight between that of glycoproteins I and II (refs 8 and 9). It is proposed that this component, which stained for carbohydrate, albeit poorly, be termed glycoprotein IIa while the former glycoprotein II be termed glycoprotein IIb. Figure 1 shows the iodination patterns from two normal donors.

The platelets from three patients³ fulfilling the diagnostic criteria of Glanzmann's thrombasthenia were iodinated with ¹²⁵I (CEA, Saclay, France), solubilised and mixed with solubilised normal platelets previously labelled with ¹²⁵I. The mixtures were reduced with 2% 2-mercaptoethanol and submitted to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis². The iodination patterns of the platelets from the three patients were almost identical and revealed marked differences from normal in the glycoprotein region. Figure 1a shows the iodination patterns obtained after coelectrophoresis of normal platelets with those from one of the patients. These patterns revealed an increase in the label associated with a polypeptide with an apparent molecular weight similar to glycoprotein I, a normal label associated with glycoprotein IIa and a decreased label associated with glycoproteins IIb and III. In addition, the molecular weight of thrombasthenic glycoprotein III was less than that of normal glycoprotein III. The ratio of the label associated with the glycoprotein region of thrombasthenic platelets from the three patients studied was 1.5:1:0.4:0.5, whereas that associated with the glycoproteins of normal platelets was 1:1:1:3.

The plasma membranes from iodinated thrombasthenic platelets were isolated by the glycerol-lysis technique¹². Thrombasthenic platelets lysed equally as well as normals and gave yields of plasma membranes within the normal range. Isolated membranes were then coelectrophoresed with normal iodinated platelets (Fig. 1b). A difference was observed between the iodination patterns of thrombasthenic platelets and isolated membranes from these platelets. Comparison of Fig. 1a and b shows that the labelled component of thrombasthenic platelets with an apparent mole-

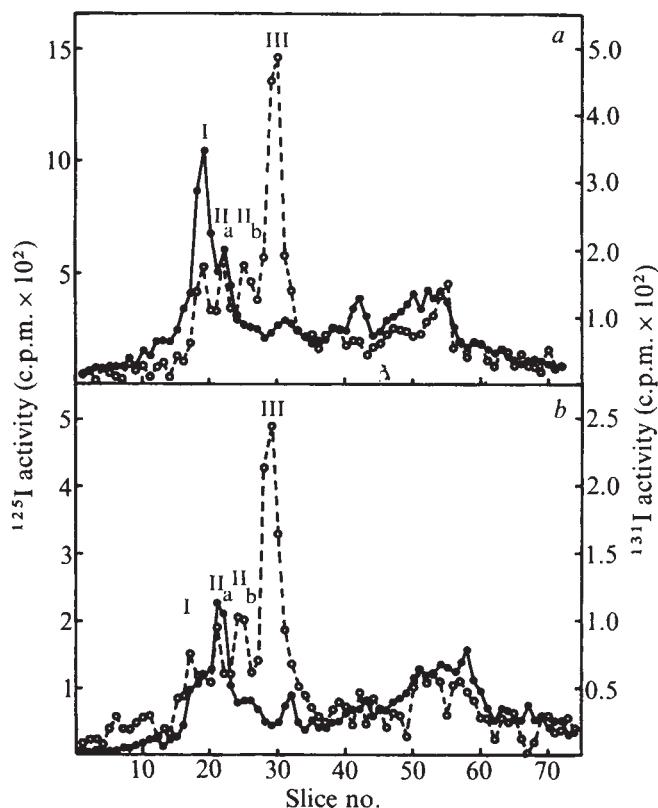


Fig. 1 Comparison of the exposed polypeptides and glycoproteins of the plasma membranes of normal platelets and platelets from a patient with Glanzmann's thrombasthenia. Blood from a normal donor and the patient were anticoagulated using ACD (acid citrate dextrose)¹⁰ and the platelets were isolated and washed by the method of Massini and Lüscher¹¹. Washed normal platelets (1 ml, 10^9 platelets) were labelled with ^{125}I whereas the washed platelets from the patient (1 ml, 10^9 platelets) were labelled with ^{125}I by the lactoperoxidase technique². Labelled platelets were washed twice and solubilised in 2–3% SDS. Labelled membranes from the patient's platelets were also isolated¹² and solubilised in 2–3% SDS. Samples labelled with ^{125}I were mixed with those labelled with ^{131}I . These mixtures were treated with 2% 2-mercaptoethanol, heated to 100 °C for 3 min, and submitted to electrophoresis on 5% polyacrylamide gels, sliced laterally into 1.6-mm disks and the radioactivity associated with each disk determined². a, ^{125}I -labelled thrombasthenic platelets (●) and ^{131}I -labelled normal platelets (○); b, ^{131}I -labelled normal platelets (○) and plasma membranes from ^{125}I -labelled thrombasthenic platelets (●).

cular weight similar to that of glycoprotein I was not isolated with the plasma membranes. Further analysis revealed that this component was also not present in the residue fraction, but appeared in the supernatant. Normal glycoprotein I, however, is isolated with the plasma membrane fraction in a concentration similar to that present in whole platelets². It therefore seems that the labelled component in the glycoprotein I region of thrombasthenic platelets, which is absent on normal platelets, is not glycoprotein I but rather a component bound to the thrombasthenic membrane surface. The significance of the decreased label associated with thrombasthenic glycoprotein I is not clear since gels stained for carbohydrate of isolated thrombasthenic membranes (not shown) revealed that the concentration of glycoprotein I was within the normal range.

Another difference between normal and thrombasthenic platelets is the decreased iodination of glycoprotein IIb on the thrombasthenic platelet membrane. Nurden and Caen⁷ concluded from studies of gels of crude thrombasthenic membranes stained with periodic acid-Schiff that glycoprotein II (glycoprotein IIb) was absent. Staining of gels

for carbohydrate, however, cannot detect low concentrations of this component. In the study reported here, because of the greater sensitivity of the iodination technique, the amount of label associated with glycoprotein IIb was 40% of that present on the normal platelet membrane. We suggest therefore that the phenotypic expression of thrombasthenia is not an absence of glycoprotein IIb but rather a reduction in its concentration.

The study reported here demonstrates that glycoprotein III is also abnormal in Glanzmann's thrombasthenia. Nurden and Caen's results⁷ indicated that thrombasthenic glycoprotein III had a lower apparent molecular weight but that its concentration was similar to that of normal platelets. The data from the iodination experiments reveal that the decreased molecular weight of thrombasthenic glycoprotein III has drastically altered its expression on the membrane surface. The decreased iodination of thrombasthenic glycoprotein III may result from either an altered primary structure which could affect its orientation in the membrane or from a missing peptide containing a tyrosine residue which is iodinated in normal glycoprotein III. Since an additional component is found on the thrombasthenic platelet membrane surface it is possible that it sterically hinders the iodination of glycoprotein III.

Platelets from patients with Glanzmann's thrombasthenia do not aggregate to adenosine diphosphate (ADP), collagen or thrombin, even though collagen and thrombin induce thrombasthenic platelets to undergo a release reaction similar to normal platelets^{3–6}. Although ADP does not induce aggregation of these platelets, it does stimulate the platelet shape change⁴. Therefore, it seems that receptors for ADP, collagen and thrombin are present on the thrombasthenic platelet membrane but that these platelets lack an essential component necessary for normal aggregation. Since platelet aggregation involves interactions between membrane surfaces, the differences in the surface proteins of thrombasthenic platelets seen in our study could account for the absence of aggregation in this disorder and may indicate those components involved in normal ADP-induced aggregation.

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Actin-tubulin homology revisited

THERE seems to be a principle underlying all eukaryotic motile systems. Movement, whether it be muscular, ciliar, flagellar or cytoplasmic, is apparently realised through the ATP-dependent sliding interaction of fibrillar proteins. This has raised the controversial question of whether the corresponding proteins from different systems are in fact homologues. Indeed, many proteins involved in motility have been isolated and characterised and have been reported as actins¹⁻³.

Evidence has been presented for^{6,7} and against^{8,9} the concept of homology between actin and tubulin, the monomeric unit of microtubules. (We use 'homology' in the sense defined by Stephens⁸.) Puszkun *et al.*^{10,11} demonstrated that colchicine-binding protein isolated from porcine brain¹⁰ and blood platelets¹¹ can stimulate ATPase activity of myosin in much the same way as actin. Others however, have shown large quantities of actin-like protein in these tissues^{12,13}, and so the results of Puszkun *et al.* might have been affected by contaminating actin.

The outer fibres of the ciliary axoneme provide an ideal tool for corroborating Puszkun and Berl's findings for large quantities of pure tubulin can be obtained easily⁶. There is no evidence that cytoplasmic actin is present in cilia of *Tetrahymena pyriformis*¹⁴.

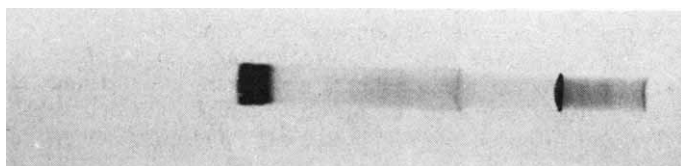
Cilia of *T. pyriformis* (W strain) were isolated and purified as before⁶. Outer fibres were prepared by dialysing demembrated axonemes against a low ionic strength buffer (1 mM Tris-thioglycollate, 0.1 mM EDTA). The outer fibre fraction was dissolved in a solution containing the detergent Sarkosyl (0.5% Sarkosyl; 10 mM imidazole-HCl, pH 6.8; 100 mM KCl; 0.2 mM GTP and 0.1% mercaptoethanol) followed by Sephadex G-25 column chromatography to remove unbound detergent. The homogeneity of the preparation was determined by polyacrylamide gel electrophoresis¹⁵.

Chicken breast myosin was isolated by the method of Richards *et al.*¹⁶. The concentration of both tubulin and myosin was determined by the technique of Lowry *et al.*¹⁷, using bovine serum albumin as a standard. The ATPase activity of myosin, in both the presence and absence of outer fibre tubulin, was measured using the Fiske-Subba Row¹⁸ assay for inorganic phosphate (for details see Table 1).

The results are shown in Table 1 and Figs 1 and 2. Figure 1 illustrates the homogeneity of the preparation, for essentially only the two bands of A and B tubulin are present in the stained polyacrylamide gel. Table 1 summarises the effect of the addition of tubulin on the Mg²⁺ ATPase activity of myosin. Activity increased about 3.8 ± 0.4 times. The tubulin-myosin ratio was not critical; optimal stimulation of activity was at a ratio of 3:1 or greater. The trace amount of ATPase activity associated with the tubulin fraction probably represents residual dynein¹⁹.

The effect of changes in the pH of the reaction mixture

Fig. 1 Polyacrylamide gel electrophoresis of outer fibre tubulin from the cilia of *Tetrahymena*. The protein was reduced and alkylated as before⁶, and run in 5% acrylamide gels with 8 M urea¹⁵. Approximately 70 µg of protein were loaded per gel.



on the tubulin stimulation of myosin is illustrated in Fig. 2. The pH curve for the interaction shows an optimum at 6.0, decreasing sharply on either side (Fig. 2), thus emphasising the importance of pH in the tubulin-myosin interaction.

These results essentially agree with those of Puszkun *et al.*^{10,11}, but differ in one important aspect. They reported more than 20-fold stimulation of myosin activity with porcine brain¹⁰ and platelet¹¹ tubulin at pH 6.8 whereas we found a roughly two-fold stimulation at that pH (Fig. 2). An explanation for the lower stimulation may be a partial denaturation of the ciliary tubulin by Sarkosyl. Data indicate that muscle actin solubilised in Sarkosyl loses 70% of its ability to stimulate the ATPase activity of myosin

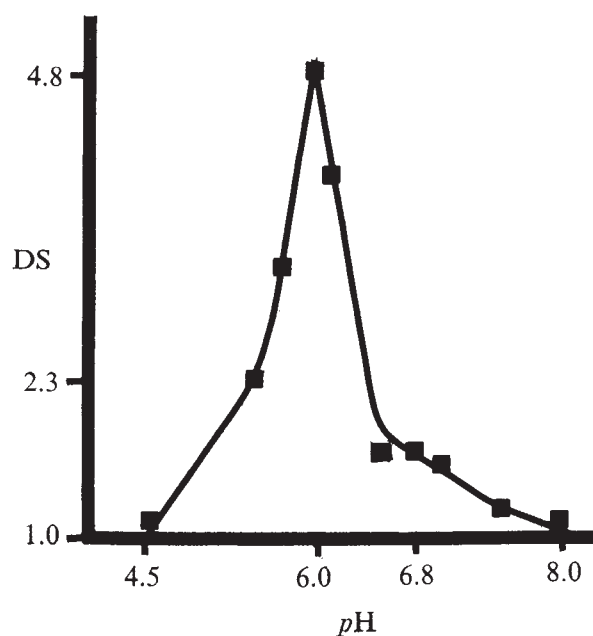


Fig. 2 Effect of pH on the stimulation of myosin ATPase by outer-fibre tubulin. The assay was performed as described in Table 1, except that the buffer used for pH values above 7 was Tris-HCl. Each point represents the average of at least two experiments. The degree of stimulation (DS) was calculated from the following formula: $DS = (MT - T)/M$ where M , T , and MT represent the specific activities of myosin, tubulin and myosin plus tubulin respectively. Note that a DS value of 1.0 represents no stimulation.

(unpublished work). On the other hand, a survey of the literature indicates that actin from different sources stimulates myosin ATPase activity to different degrees^{3,5}. If this is taken into consideration the value we obtained is comparable with that reported for the stimulation of muscle myosin by *Acanthamoeba* actin³ where the stimulation was approximately fourfold.

The optimal pH of the interaction (6.0) agrees rather well

Table 1 Stimulation of Mg²⁺ ATPase activity of muscle myosin by ciliary outer fibre tubulin.

Protein sample	Specific activity (µmol Pi per min per mg protein)
Myosin	0.028 ± 0.06
Tubulin	0.005 ± 0.01
Myosin and tubulin	0.097 ± 0.05

The reaction mixture consisted of 30 mM imidazole-HCl buffer, pH 6.0, at 25 °C; 0.06 M KCl; 0.5 mM ATP and 1 mM MgCl₂. Each tube contained 2 ml of reaction mixture plus 40 µg of myosin and/or 114 µg of tubulin. Duplicate samples were incubated in a water bath and stopped after 0 and 30 min incubation by the addition of 0.5 ml of sodium dodecyl sulphate, followed by 0.25 ml of molybdate reagent and 0.15 ml of the Fiske-Subba Row reagent. The tubes were then read at 660 nm with a Zeiss PMQ-11 spectrophotometer.

with the reported pK of histidine. This suggests that histidine and its methylated derivative in actin could play a role in the stimulation of the myosin ATPase activity by both actin and tubulin. This is supported by the finding that histidine is essential for the polymerisation of G-actin²⁰ together with the report that polymerisation is characteristic of the interaction between G-actin and myosin²¹.

The idea of homology between actin and tubulin arose from the finding that the amino acid composition and molecular weight of tubulin closely parallel those reported for actin⁶. Other workers questioned this homology. Mohri and Shimomura reported no stimulation of myosin by sea urchin sperm flagella⁹. However, they solubilised the tubulin in thermal conditions that could result in the denaturation of the protein²². Stephens⁸ bases his objections to the homology on the fact that the newer determination of the molecular weight of actin²³ does not agree with that established for tubulin. Indeed, he shows that the peptides produced by tryptic and chymotryptic digests of tubulin and actin isolated from *Pecten irradians* are dissimilar. There may however, be limited regions in the primary and tertiary structure of tubulin and actin that are similar, which could explain the stimulation of myosin by tubulin.

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Cross innervation and the regulatory protein system of rabbit soleus muscle

SINCE Buller *et al.* demonstrated¹ that the contraction speed of skeletal muscle is determined by the nature of its innervation, there have been several reports that the biochemical composition and properties of muscle are likewise influenced. When the motor nerve to a slow muscle is replaced by a nerve that normally innervates a fast muscle, changes in phosphorylase, succinic dehydrogenase and myosin ATPase activities, such as would be expected if the biochemical characteristics of the fibres were changing from those of type I to those of type II, can be demonstrated histochemically². This is also true in the opposite situation, when the nerve to a fast muscle is replaced with one that normally innervates a slow muscle. The histochemical evidence implies that a change in concentration of the enzymes studied has occurred in response to the new type of innervation and although it would not be evident in such investigations, the possibility of change in isoenzyme complement cannot be excluded. In the case of myosin, however, which has been isolated after cross innervation, the changes

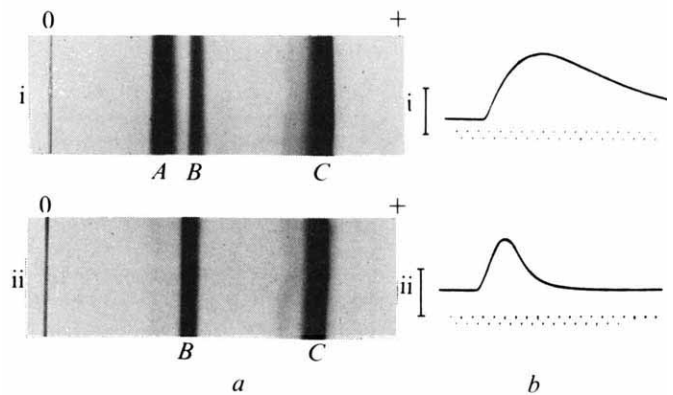


Fig. 1 Effect of cross innervation on the speed of contraction and the troponin I composition of rabbit soleus muscle. *a*, Electrophoresis of troponin I (20 µg), isolated by affinity chromatography from rabbit soleus muscle (rabbit 2, see Table 1), in the presence of troponin C (50 µg) from rabbit white skeletal muscle. 6 M urea, 25 mM Tris–80 mM glycine, pH 8.6, 8% acrylamide. O, Origin. i, Control soleus; ii, soleus from the other leg of the same animal 26 weeks after cross innervation. A, slow troponin I-troponin C complex; B, fast troponin I-troponin C complex; C, troponin C. *b*, Records of single isometric contractions of the soleus muscles used for troponin I preparations analysed in *a*. The distance between successive dots represents 10 ms. Vertical scale represents 100 g tension.

have been analysed with more precision. These indicate that when the contraction speed of skeletal muscle is altered by either cross innervation or long term electrical stimulation, there are changes in V_{max} of the myosin ATPase, in its light chain components and in its electrophoretic mobility^{3–7}. These changes imply that new myosin molecules of different primary and quaternary structure are being synthesised as a consequence of the altered activity pattern of the muscle resulting from these procedures. They correlate well with the widely accepted view that the V_{max} of the myosin ATPase rises with increasing muscle speed⁸ and the differences in light chain components known to exist in myosin from fast and slow skeletal muscle^{9–12}.

Biochemical investigations carried out so far on cross-innervated muscles have been restricted to enzymes involved in energy production and utilisation. As there is now good evidence that tropomyosin¹³ and the components of the troponin system differ in fast and slow skeletal muscle (see ref. 14 for review), we have investigated the effect of cross innervation on those proteins of the I filament which are involved in the regulation of the actomyosin ATPase.

Cross innervation of the soleus muscle was performed in 13 adult New Zealand Red rabbits under Nembutal anaesthesia. Using full aseptic precautions, the nerve to the soleus muscle in one leg was sectioned and sutured to the medial head of the gastrocnemius muscle to prevent self reinnervation. The nerve to the fast muscle, the lateral popliteal, was cut close to its point of entry in to the tibialis anterior muscle and sutured to the soleus. Between 2 and 6 months later, the soleus muscles of both operated and control legs were prepared for the recording of isometric tension¹⁵. Control muscles were stimulated by way of the cut peripheral end of the medial popliteal nerve with square-wave pulses of supramaximal intensity. Contractions of the cross-innervated muscles were elicited by stimulation of the lateral popliteal nerve. The medial popliteal nerve was also stimulated to assess whether some portion of the muscle had become self reinnervated. Times to peak tension of both muscles were measured from records of single twitches. When measurements were complete the muscles were frozen at -20°C and troponin I or tropomyosin subsequently isolated from both control and cross-innervated muscles.

For preparation of troponin I the muscles were homo-

Table 1 Effect of cross innervation on the relative proportions of the polymorphic forms of troponin I and tropomyosin in rabbit soleus muscle

Animal no.	Weeks after cross innervation	Cross innervated soleus					Control soleus			
		Contraction time (ms) X Own nerve*	Tropomyosin α/β (molar ratio)	Troponin I		Contraction time (ms)	Tropomyosin α/β (molar ratio)	Troponin I		
				Fast (%)	Slow (%)			Fast (%)	Slow (%)	
9	8	62	—	—	50	50	70	—	31	67
8	8	32	—	—	73	27	61	—	24	76
3	22	32	63	—	16	84	54	—	30	70
5	23	29	—	—	77	23	75	—	36	64
2	26	26	—	—	97	3	52	—	37	63
1	27	25	—	—	79	21	54	—	27	73
10	12	31	—	1.10	—	—	60	1.04	—	—
11	13	44	61	1.07	—	—	54	0.98	—	—
12	19	30	—	1.06	—	—	70	1.00	—	—
14	20	30	—	0.96	—	—	80	1.03	—	—

*The absence of figures in this column indicates that reinnervation by the muscle's own nerve did not occur.

Relative amounts of the fast and slow forms of troponin I and of the α and β subunits of tropomyosin were determined by comparing the intensities of staining with Coomassie brilliant blue of electrophoretic bands, using a Unicam SP500 spectrophotometer coupled to a Gilford linear transporter absorbance indicator. Electrophoresis of troponin I carried out in the conditions described in text and in Fig. 1; tropomyosin applied to a 10% (w/v) polyacrylamide gel with 0.1% SDS, 6 M urea, and 82.5 mM Tris-400 mM boric acid buffer, pH 7.0 (ref. 13).

genised in 20 vol of a solution containing 9 M urea, 1 mM CaCl_2 , 15 mM 2-mercaptoethanol, 75 mM Tris, adjusted to pH 8.0 with 1 M HCl, and applied to a Sepharose-troponin C affinity column as described¹⁶. After washing well with buffer and subsequent application of the same buffer in which the CaCl_2 was replaced by 10 mM EGTA, troponin I was eluted from the column free from other proteins.

Two polymorphic forms of troponin I are present in rabbit skeletal muscle. These are fast skeletal troponin I, which is the only form present in psoas and longissimus dorsi muscle¹⁶ and slow skeletal troponin I, which is the principal form present in red skeletal muscles such as the soleus and crureus. The two forms have different primary structures but migrate with identical mobilities on electrophoresis in SDS. Although neither form of troponin I migrates to the anode on electrophoresis in 6 M urea, 25 mM Tris-80 mM glycine, pH 8.6, the complexes formed by the two forms with troponin C in the presence of Ca^{2+} in these conditions show electrophoretic mobilities sufficiently different to allow them to be distinguished readily and their relative amounts estimated (Fig. 1). Control experiments using a sample of pure fast skeletal troponin I to which 3-4 times a molar excess of troponin C isolated from white rabbit skeletal muscle was added, showed that all the troponin I present in the mixture moved as the complex on electrophoresis in 6 M urea, pH 8.6. Thus by densitometric scanning of the bands of the complexes formed on electrophoresis when the total troponin I extracted from the muscle was mixed with excess troponin C, an estimate of the relative amounts of the two forms of troponin I present could be made. The validity of this procedure to within $\pm 3\%$ was confirmed by control experiments in which samples of troponin I of known composition were applied to the gel. In this way, we have shown that the troponin I of normal New Zealand Red rabbit soleus consists of about 30% fast and 70% slow skeletal troponin I.

In all cases in which the contractile speed of the soleus muscle increased after cross innervation, a corresponding change in the proportion of fast and slow troponin I was observed. On average, 22-27 weeks after cross innervation, the fast skeletal troponin I increased from 30% to more than 70% of the total troponin I extracted by affinity chromatography. In one experiment the troponin I present in the soleus was converted almost completely to the fast skeletal muscle type (Fig. 1 and Table 1, animal no. 2). Where physiological measurements showed that most of the muscle had become reinnervated by its own nerve (Table 1, animal no. 3), the speed of contraction remained slow

and there was no increase in the relative amount of fast troponin I. The change in the proportion of the two forms of troponin I seemed to precede the change in contractile speed in one animal (Table 1, animal no. 9) that was examined 8 weeks after the operation. This observation is being investigated further.

The form of troponin I that increased in amount was not a simple modification of the slow muscle type but represented new protein synthesis for the electrophoretic pattern of the cyanogen bromide digest was similar to that previously described for fast skeletal muscle troponin I of the rabbit and different from that of the cyanogen bromide digest of slow skeletal troponin I (ref. 16).

Tropomyosin was isolated from soleus muscles frozen immediately after measurement of the speed of contraction by the method of Cummins and Perry¹³. The protein was separated into the α and β components by electrophoresis, the bands stained with Coomassie brilliant blue and the relative amounts present determined by densitometric scanning¹³. A molar ratio of α and β subunits in soleus muscle of the New Zealand Red rabbit of 1.01 was obtained, which is similar to that reported for the New Zealand White rabbit¹³. The ratio did not change significantly 3-6 months after cross innervation, when the contraction time had increased by a factor of approximately two and marked changes in the troponin I composition had occurred (Table 1).

The evidence indicates that when the contractile speed of soleus muscle is increased by replacing its normal nerve supply by a nerve that usually supplies a fast muscle, the slow skeletal troponin I (which is the major polymorphic form of the protein present in whole soleus muscle) is replaced by fast skeletal muscle troponin I. If no changes in the relative rates of catabolism of these two proteins occur this implies that the change in innervation has altered the relative rates of synthesis of the two forms of troponin I, which, from their primary structural differences¹⁶, would be presumed to be under different genetic control.

Although the molar ratios of α and β subunits of tropomyosin in the muscles of the rabbit roughly correlate with the speed of contraction¹³ there was no change in this ratio during similar periods of cross innervation. Troponin C was not examined after cross innervation for there is evidence that this protein is very similar, if not identical, in fast and slow skeletal muscle^{17,18}. Neither was troponin T studied although different forms of this protein exist in the red and white skeletal muscle of the chicken^{19,20}. We conclude that the type of innervation markedly affects the polymorphic form of troponin I in the muscle, but not the

tropomyosin and probably not the troponin C. This implies that different factors are involved in regulating the genes concerned in the synthesis of the individual proteins of the regulatory system.

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New peptide in the vertebrate CNS reacting with antigastrin antibodies

RELEASING factors¹ and other brain peptides² have been the subject of extensive studies and the high sensitivity of modern measurement techniques has led to the discovery, in various tissues^{3,4} and in the nervous system⁵⁻⁹, of new, unpredicted locations for otherwise well known peptides. Evidence for the presence in the vertebrate nervous system of a hitherto undescribed brain gastrin immunoassayable peptide (BGP) is presented here.

Various tissues and regions of the central nervous system (CNS) were studied using human material obtained 24 h after death and animal material was dissected immediately after decapitation. All specimens were inspected under the microscope for normality. For extraction of immunoassayable material, a 10% w/v suspension of fresh tissue in a buffer of potassium phosphate (0.05 M) and sodium chloride (0.15 M) at pH 7.4 was homogenised at 4 °C with a glass homogeniser and a motor-driven Teflon pestle. Samples (2 ml) of homogenate were used for dry weight analysis and the rest was boiled for 15 min and then centrifuged at 100,000g for 30 min. Samples of supernatant were taken for estimation of Lowry-positive material¹⁰ and of gastrin immunoassayable material using a technique with antigastrin antibodies of high specificity¹¹. Limits of detection were 15 or 60 pg Gastrin 2-17 (hexadecapeptide, ICI) per ml in 200-μl or 50-μl samples, respectively.

To obtain a dose-response curve, Gastrin 2-17 and boiled extract of human cortical grey matter concentrated after lyophilisation were used. For standard curve establishment, Gastrin 2-17 was diluted in a buffer of potassium phosphate (0.01 M) and sodium chloride (0.15 M) at pH 7.4 containing 10 mg per ml egg albumin (Sigma), or in human cortical grey matter boiled extract from which immunoreactivity had or had not been removed by charcoal absorption¹².

Our results for gastrin in blood serum are in agreement with those published¹³⁻¹⁵ and similar results were obtained for

gastric antrum^{16,17}, obtained from autopsy material. Distribution of BGP is otherwise restricted to the nervous system (Table 1). Highest concentrations are found in cortical grey matter of all cerebral lobes; concentration in the hypothalamus is much lower and similar to concentrations of brain stem and spinal cord. BGP was not detectable, in the peripheral nervous system, in cerebellum nor in any invertebrate nervous system studied so far (Table 2).

Dose-response curves (Fig. 1) with unlabelled Gastrin 2-17 are different from those obtained with successive dilutions of concentrated boiled cortical grey matter extract. When Gastrin 2-17 is diluted in boiled brain extract from which immunoreactivity has been removed by charcoal absorption¹² the dose-response curve is very similar to that obtained with regular diluant. When Gastrin 2-17 is added to immunoreactive boiled brain extract, a further decrease in the bound-to-free radioactivity ratio was obtained. On the contrary, however, the slope of the dose-response curve is less accentuated with concentrated boiled cortical grey matter extract, than with Gastrin 2-17, and with the highest concentrations the bound-to-free ratio rests near 0.10. These differences in dose-response curves were observed with two different rabbit antigastrin sera.

Following Feldman and Rodbard¹⁸, we can use the equation

$$R^2 + R(P'K + PK - QK + 1) - QK = 0 \quad (1)$$

(*R* is the bound-to-free ratio, *P'* the concentration of labelled Gastrin 2-17, *P* the concentration of unlabelled Gastrin 2-17, *Q* the concentration of antigastric antibodies sites, and *K* the antibody affinity constant at equilibrium for Gastrin 2-17) to calculate from the dose-response curve an affinity constant for Gastrin 2-17 of our antibody of 4.9×10^{10} l per mol Gastrin 2-17. This figure is similar to the values obtained by Rehfeld *et al.*¹⁸ which are between 0.5×10^{10} and 100×10^{10} l mol⁻¹ Gastrin 2-17. Similar calculations with BGP dose-response curve lead to an affinity constant of 0.9×10^{10} l mol⁻¹, showing clearly that BGP and Gastrin 2-17 are not identical competitors ($K_{\text{gastrin}} = 5.4 \times K_{\text{BGP}}$) with labelled Gastrin 2-17 for binding our antigastrin antibody. Whether these differences in affinity and the plateau obtained with highest concentrations of BGP are a result of the presence of different antigastrin antibodies at different concentrations, some reacting with Gastrin 2-17 and BGP, some reacting only with Gastrin 2-17, remain to be studied.

Disappearance of immunoreactivity of BGP, gastric antrum extract and Gastrin 2-17 was obtained with incubation at 37 °C and pH 7.4 for 30 min with Pronase E (70,000 PUK g⁻¹, Merck) 1 mg ml⁻¹ or chymotrypsin A₄ (für analytische Zwecke, Boehringer) 1 mg ml⁻¹. With 1 mg ml⁻¹ trypsin (für analytische Zwecke, Boehringer), 70% of the immunoreactivity was preserved, as with gastric antrum extract or Gastrin 2-17. This is in accordance with the relative resistance to trypsin of Gastrin

Table 1 Distribution of gastrin immunoassayable substance in selected human tissues

Tissue	Dry weight	Lowry-positive material	Gastrin assay
Cerebral lobes	344 ± 3	21 ± 0.3	130 ± 7
Gastric antrum	302 ± 6	36 ± 1.3	14,800 ± 700
Blood serum	228 ± 3	25 ± 0.4	0.44 ± 0.05
Liver	357 ± 2	44 ± 1.1	< 3
Skeletal muscle	352 ± 6	43 ± 1.8	< 3
Myocardium	329 ± 2	41 ± 1.2	< 3
Lung	298 ± 4	47 ± 1.7	< 4
Kidney	293 ± 2	39 ± 1.1	< 4

Tissue dry weight in mg per g wet weight, boiled extract Lowry-positive material in mg per g dry weight, boiled extract gastrin immunoassayable material (gastrin assay) in ng per g dry weight. Means and standard errors of the mean (± s.e.m.) for five separate (five autopsies) determinations are presented for each tissue. Appropriate dilutions to perform gastrin radioimmunoassay on samples with an immunoreactivity below 80 pg gastrin per 50-μl or 200-μl sample.

Table 2 Distribution of gastrin immunoassayable substance in nervous system of human and animals

Human	Dry weight	Gastrin assay	Animal	Dry weight	Gastrin assay
Central nervous system			Vertebrates		
Cortical			Mammals: Dog		
grey matter	317±1	199±7	Hemisp. brain	372±2	169±6
Cortical			Cerebellum	360±2	<0.7
white matter	372±4	3±0.25	Birds: Pigeon		
Lenticular			Hemisp. brain	338±4	130±7
nucleus	339±6	26±8	Cerebellum	351±5	<0.7
Cerebral lobes	344±3	130±7	Fish: Trout		
Hypothalamus	327±4	6±0.6	Hemisp. brain	348±4	130±6
Brain stem	360±4	8±0.6	Amphibian: Frog		
Cerebellum	330±2	<0.7	Hemisp. brain	340±4	125±5
Spinal cord	420±5	4±0.7			
Peripheral nervous system			Invertebrates		
Spinal ganglia	366±5	<0.7	Molluscan—Loligo	—	<0.5*
Sympathetic			Cerebroid		
ganglia	355±7	<0.7	Ganglia		
Peripheral roots	295±6	<0.7	Crustacean—Homarus	—	<0.5*
Peripheral			Ventral		
nerves	390±6	<0.7	Ganglia		

Tissue dry weight in mg per g wet weight, boiled extract gastrin immunoassayable material in ng per g dry weight. Means and standard errors of the mean ($\pm$ s.e.m.) for three separate (three autopsies or three animals) determinations are presented. Appropriate dilutions to perform gastrin radioimmunoassay (gastrin assay) on samples with immunoreactivity below 80 pg gastrin per 50- μ l or 200- μ l sample.

* ng per g net weight.

2–17 previously reported²⁰. Disappearance of immunoreactivity of BGP after action of proteolytic enzymes, resistance of BGP to boiling for 15 min at 100 °C and its absorption on charcoal favour a small peptide molecule. Preliminary results with Sephadex G25 (columns 2.8 cm in diameter and 45 cm long) show that BGP is eluted between Gastrin 2–17 and ¹³¹I. This sets the molecular weight of BGP to less than that of Gastrin 2–17 (2,020).

BGP is different from releasing factors in that its highest concentration is found in the cortex. Of other natural substances related to gastrin by amino acid composition, cholecystokinin-pancreozymin and caerulein²¹, none has been described previously in cortical grey matter. The discovery in the vertebrate CNS of a new peptide reacting with gastrin antibodies is not unexpected in view of the postulated neural crest origin of the endocrine polypeptide (APUD) cells of the

gastrointestinal tract and pancreas²². The interest in brain-restricted peptides is that they are likely candidates for involvement in the function of the nervous system. At a time when the brain is increasingly being considered not only as a tissue with synaptic connections but also as an "endocrine organ and hormone target"²³, such a new peptide takes on an even greater significance.

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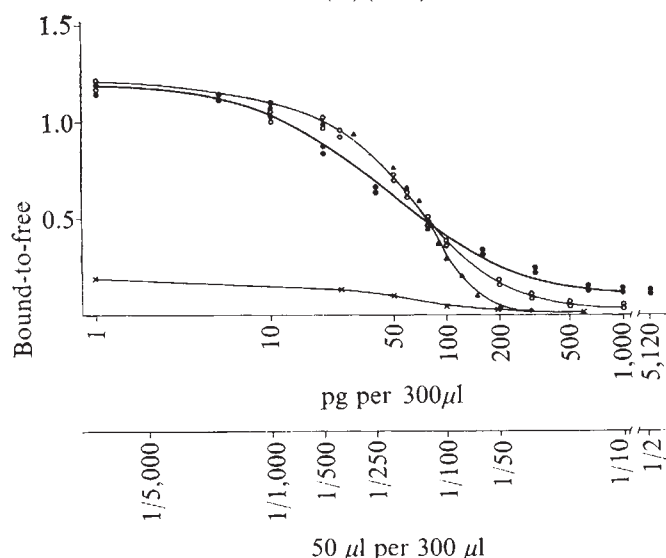
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Fig. 1 Radioimmunoassay for Gastrin 2–17. Bound-to-free ratio of labelled Gastrin 2–17 plotted against log dose of pg gastrin activity of solution of Gastrin 2–17 in a buffer of potassium phosphate (0.01 M), sodium chloride (0.15 M) at pH 7.4 with 10 mg ml⁻¹ egg albumin (○) in boiled brain extract from which immunoreactivity has (△) or has not (×) been removed by charcoal and of various dilutions of concentrated boiled brain extracts (●) (BGP).



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Role of sodium in neuronal uptake of monoamines and amino acid precursors

It is generally accepted that the neuronal re-uptake of monoaminergic neurotransmitters after their release into the synaptic cleft, inactivates transmitter action^{1,2}. The amino acid precursors L-tyrosine and L-tryptophan are also taken up by neurones from the circulation³⁻⁵. Using *in vitro* models of these processes, it has been demonstrated that tricyclic antidepressant drugs inhibit the uptake of both the amines^{6,7} and their precursors⁸ into synaptosomal fractions obtained from rat brain homogenates. Amino acids can be transported into cells by an Na⁺-dependent, Na⁺-independent or an Na⁺-inhibited transport system⁹. Monoamines, however, have been reported^{10,11} to have only an Na⁺-dependent system. Since information is lacking concerning the uptake of L-tyrosine and L-tryptophan, we have attempted to determine the role of Na⁺ ions in the uptake of the amino acid precursors compared with that of monoamines.

The uptake of the amino acids ¹⁴C-L-tyrosine and ¹⁴C-L-tryptophan and their related monoamines ¹⁴C-1-noradrenaline (NA), ¹⁴C-dopamine (DA) and ¹⁴C-5-hydroxytryptamine (5-HT) was studied in a crude synaptosomal fraction obtained from rat brain homogenate. The preparation of synaptosomal fractions and the methods used for the uptake experiments have been described⁸ (see legend to Fig. 1 for details). After incubation of the amino acids the pellet did not contain measurable amounts of formed monoamines (unpublished results and ref. 12). No attempt was made to separate the ¹⁴C-monoamines from metabolites formed, but, to prevent degradation of the monoamines by monoamine oxidase (MAO) an MAO-inhibitor (nialamide) was added to the incubation fluid⁶. The samples were centrifuged after incubation for 30 min at 70,000g, the supernatant discarded and the pellet rinsed twice with cold buffer. The pellet was dissolved in 1.0 ml deionised water by ultrasonic disintegration (5 s) and then 12 ml Instagel was added. Radioactivity was measured with a Packard liquid scintillation spectrometer.

Figure 1 shows the uptake of both amino acids by synaptosomes incubated in Krebs-phosphate buffer containing different Na⁺ concentrations and it can be seen that replacement of Na⁺ by sucrose resulted in an increased uptake. Thus, increasing the Na⁺ concentration inhibits the uptake of amino acid, but the uptake of the three monoamines studied is enhanced when the Na⁺ concentration is increased (Fig. 1b). Although it is known that the uptake of NA and 5-HT is Na⁺-dependent^{10,11}, a small amount is taken up in the absence of Na⁺. An effect of sucrose on the uptake processes seems rather unlikely since replacement of sodium by choline produced the same effects as replacement by sucrose (unpublished). Our results show that uptake of 5-HT was maximal at 50 mM Na⁺, whereas the uptake of NA and DA was maximal at about 100 mM Na⁺. *In vitro* conditions can, therefore, be created in which the uptake of 5-HT will be nearly maximal (25 mM Na⁺) without affecting the uptake of NA or DA. Optimal conditions for the uptake of NA, DA and 5-HT differ considerably from those for tyrosine and tryptophan.

Transformation of these results into a physiological model suggests that the re-uptake of monoamines and the uptake of amino acid precursors into nerve terminals is regulated by cation fluxes which are also responsible for the generation and conduction of impulses. During depolarisation, Na⁺ influx is increased and so an Na⁺ deficiency near the extracellular site of the axonal membrane may ensue. This Na⁺ deficiency will promote the uptake of tyrosine into the nerve ending, while at the same time NA or DA will be released into the synaptic cleft as a consequence of an increased influx of Ca²⁺ ions during depolarisation¹³⁻¹⁵. Both the increased intraneuronal tyrosine concentration and the release of NA

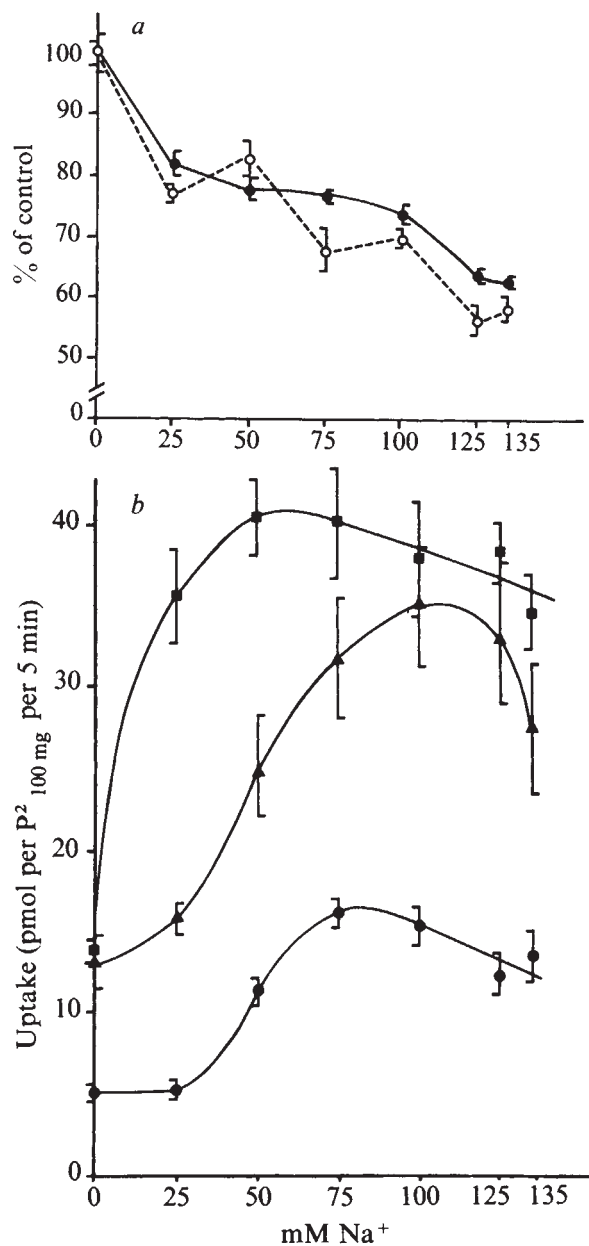


Fig. 1 *a*, Effect of Na⁺ concentration on the uptake of L-¹⁴C-tyrosine and L-¹⁴C-tryptophan by a synaptosomal fraction *in vitro*. The synaptosomal fraction was suspended in a Krebs-phosphate buffer (pH 7.4) (5 ml per g of tissue) containing (in mM): NaCl 118.46; KCl, 4.74; CaCl₂, 1.27; KH₂PO₄, 1.18; MgSO₄, 1.18; Tris-HCl, 16.15 and glucose, 40.0. For each incubation experiment 0.5 ml of the suspension, equivalent to a synaptosomal fraction from 100 mg of brain tissue, was used. Synaptosomes were preincubated for 15 min at 37 °C in a shaking incubator, and the ¹⁴C-labelled compound, dissolved in 0.5 ml Krebs buffer, was added and incubation continued for a further 5 min. Incubation was stopped by placing the incubation vessels in ice water. Buffers containing different Na⁺ concentrations were prepared by replacing NaCl isosmotically by sucrose. Uptake was measured 5 min after addition of L-¹⁴C-tyrosine (2 × 10⁻⁵ M; 3.4 mCi mmol⁻¹) or L-¹⁴C-tryptophan (0.25 × 10⁻⁵ M; 13.9 mCi mmol⁻¹). Quenching was measured by the automatic external standardisation (AES) ratio method. *b*, Effect of Na⁺ concentration on uptake of NA, DA and 5-HT by a synaptosomal fraction *in vitro*. Conditions were as described in *a* except that the incubation fluid also contained nialamide (10⁻⁴ M). Uptake was measured at bath concentrations of 5 × 10⁻⁸ M. Specific activities NA, 54 mCi mmol⁻¹; DA, 57.3 mCi mmol⁻¹; 5-HT, 54 mCi mmol⁻¹. Compounds used and specific activities: L-tryptophan (methylene-¹⁴C), 52 mCi mmol⁻¹; L-tyrosine-¹⁴C (U), 522 mCi mmol⁻¹; 5-HT-3'-¹⁴C creatinine sulphate, 55 mCi mmol⁻¹; dopamine (ethylamine-2-¹⁴C) hydrochloride, 57 mCi mmol⁻¹; 1-noradrenaline *d*-bitartrate (methylene-¹⁴C), 57 mCi mmol⁻¹ (all Radiochemical Centre, Amersham). *a*, ●, L-tyrosine; ○, L-tryptophan; *b*, ●, NA; ▲, DA; ■, 5-HT.

or DA will enhance biosynthesis of these transmitters, because more precursor is now available and the product feedback repression of tyrosine hydroxylase is removed¹⁶⁻¹⁸. Furthermore, the biosynthesis of NA or DA may be increased by the influx of both cations which have been reported to activate tyrosine hydroxylase^{19,20}.

During the restoration phase Na^+ ions are expelled into the extracellular space which induces re-uptake of monoamines. During the resting state the uptake of monoamines is thus optimal, while the synthesis and release of monoamines is inhibited. During depolarisation, however, the release and synthesis of monoamines is activated and at the same time the re-uptake of tyrosine and tryptophan is enhanced. During this phase of the action potential the re-uptake of monoamines is inhibited. This impairment of the uptake of monoamines, after their release into the synaptic cleft, potentiates the action of the released transmitter on its receptor. The same mechanism for a coupling between impulse inflow or cation fluxes and release, synthesis and re-uptake of transmitter can be applied to 5-hydroxytryptaminergic nerves, but in this case the uptake of tryptophan may be more critical for an increased biosynthesis of 5-HT since as yet no product feedback repression of tryptophan hydroxylase is known.

Since the most important assumption of this hypothesis is the extracellular deficiency of Na^+ ions near the axonal membrane during depolarisation, a theoretical calculation concerning this problem is given below. If one assumes that the area of the presynaptic membrane is $1 \mu\text{m}^2$ and the extracellular Na^+ ion concentration is 0.1 M , the number of Na^+ ions per μm^3 will be $10^{-15} \times 0.1 \times \text{Avogadro's number}$ (N). N is 6×10^{23} , so the number of Na^+ ions per μm^3 will be 6×10^7 . Assuming a uniform distribution of Na^+ ions, the number of Na^+ ions occupying $1 \mu\text{m}^2$ will be $(6 \times 10^7)^{2/3} = 15 \times 10^4 \text{ Na}^+$ ions. In non-myelinated nerve tissue each impulse will cause an influx of 20,000 Na^+ ions per μm^2 (ref. 19), so deficiency near the axonal membrane might occur if the diffusion of extracellular Na^+ is slow enough.

In cases of frequent stimulation, it is not unlikely that after seven impulses a complete layer of Na^+ ions will have disappeared. In the synaptic cleft, which has a volume of about $0.02 \mu\text{m}^3$, only eight layers of Na^+ ions are present, each consisting of 150,000 Na^+ ions. These ions not only serve to conduct the presynaptic action potential, but may also be needed for the generation of a postsynaptic action potential. Although no data are available of the diffusion of Na^+ , or other ions, near the axonal membrane or in the synaptic cleft, one might expect that in a supercapillary system such as the synaptic cleft, the pre- and postsynaptic resting potential would exert a strong influence on the displacement of ions. The negative resting potentials at the pre- and postsynaptic

site will fix the position of Na^+ ions in the synaptic cleft. During depolarisation of the presynaptic membrane, 20,000 Na^+ ions will enter the presynaptic nerve ending, resulting in a decrease of the negative potential. Therefore, during depolarisation, the remaining Na^+ ions present in the synaptic cleft will shift to the postsynaptic site, creating an Na^+ deficiency near the surface of the presynaptic membrane. Thus, supposing that during depolarisation the displacement of extracellular Na^+ ions is sufficiently slow, an Na^+ deficiency near the axonal membrane might be expected. This condition will be favourable for an increased uptake of amino acid precursors simultaneously with an inhibition of the re-uptake of the stimulus-induced released monoamines. During the restoration phase, when the efflux of Na^+ ions is increased, the re-uptake of monoamines is restored, and the biosynthesis will slow down as a result of product feedback repression and (or) an impairment of the uptake of amino acid precursor (Fig. 2). In this way synthesis, release and re-uptake of monoaminergic neurotransmitters are all coupled to cation fluxes which occur during nervous impulse flow.

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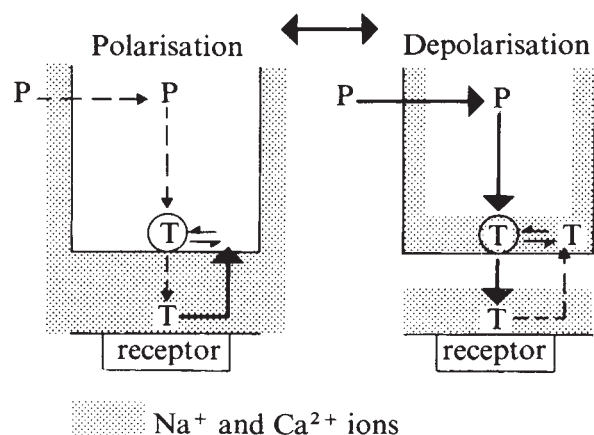
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Fig. 2 Hypothesis for the effect of impulse flow on the re-uptake, synthesis and release of monoaminergic neurotransmitters. P, Precursor; T, transmitter; ---, low activity or inhibition; —, increased activity.



Enteropancreatic circulation of digestive enzyme as a conservation mechanism

THE permeability of the intestine to pancreatic digestive enzymes has been known for some years¹⁻⁷. Recently it was demonstrated⁸ that chymotrypsinogen and amylase are transported across the pancreatic acinar cell as well. Taken together these observations suggest that some of the digestive enzymes which are secreted into the intestinal lumen are circulated by way of the bloodstream and acinar cell back into the intestine. This prediction was borne out experimentally. ³H-chymotrypsinogen instilled into the intestinal lumen reappeared in pancreatic secretion within minutes⁸. The question remained, however, whether this "enteropancreatic circulation" was a trace process either of a trivial nature or serving a regulatory function; or, whether—analogue to the enterohepatic circulation of bile salts—it was a process in which substantial amounts of digestive enzyme could be circulated and thereby conserved. The

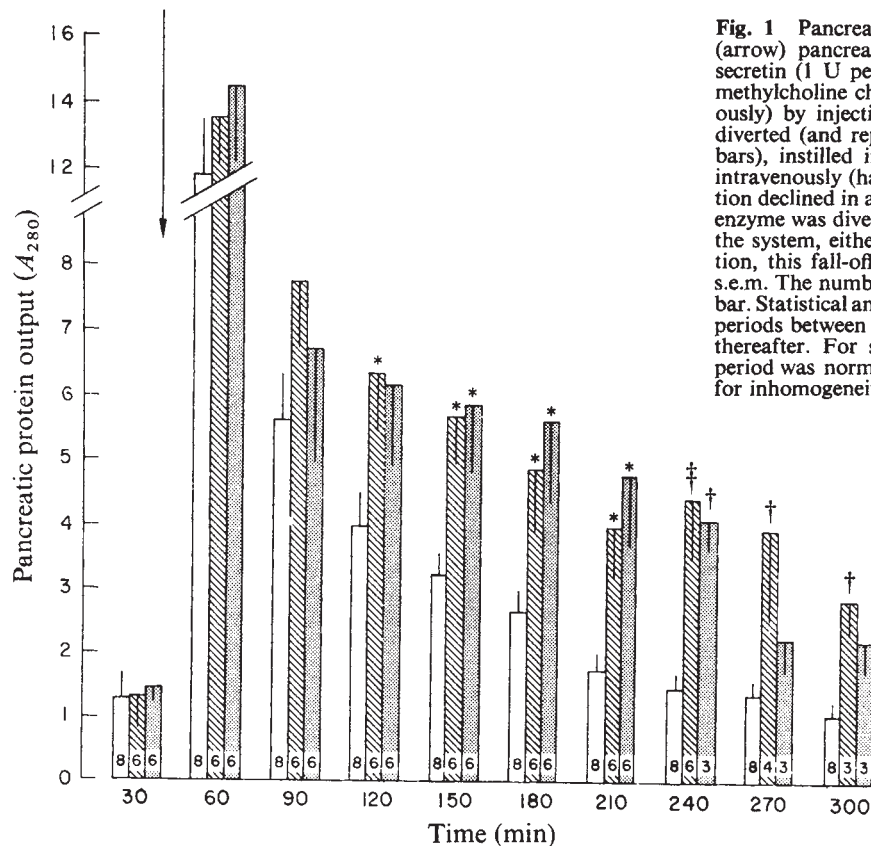


Fig. 1 Pancreatic protein secretion with time. After 30 min (arrow) pancreatic secretion was continuously stimulated with secretin (1 U per kg body weight, intravenously) and acetyl- β -methylcholine chloride (0.5 mg per kg body weight, subcutaneously) by injections every 30 min. Pancreatic juice was either diverted (and replaced by an equal quantity of albumin) (open bars), instilled into the duodenum (stippled bars), or injected intravenously (hatched bars). In these conditions, enzyme secretion declined in a roughly exponential manner when the digestive enzyme was diverted (control). When enzyme was added back to the system, either by intravenous injection or intestinal instillation, this fall-off was less pronounced. Error bars indicate the s.e.m. The number of experiments is given at the bottom of each bar. Statistical analysis was done using the Mann-Whitney test for periods between 90 and 150 min and Student's *t*-test for periods thereafter. For statistical analysis the protein output of each period was normalised for the peak response (60 min) to control for inhomogeneity of variance between experiments. * $P < 0.05$; † $P < 0.025$; ‡ $P < 0.01$.

experiments reported here are consistent with this latter view and suggest the circulation of approximately 60% of a mixture of digestive enzymes instilled into the duodenum (82–92% at steady state).

The pancreatic duct of male white New Zealand rabbits weighing 2–3 kg was cannulated *in situ* after a median laparotomy under DIAL-urethane anaesthesia (0.7 ml per kg body weight intraperitoneally) and subsequent to a 20 h fast. The continuity of the gastrointestinal lumen was maintained. Protein content was estimated by ultraviolet absorption at 280 nm. These measurements were comparable with values obtained with the Folin phenol reagent (protein content was estimated using both methods in selected experiments). After a 30-min unstimulated collection period, maximal secretion of both protein and fluid was continuously elicited by half-hourly injections of acetyl- β -methylcholine chloride (0.5 mg per kg body weight subcutaneously) and porcine secretin (1 Ivy dog unit per kg body weight intravenously) (kindly provided by Professor V. Mutt of the Karolinska Institutet). Samples were collected at 30-min intervals.

When pancreatic juice was completely diverted from the intestine, a roughly exponential decay in protein secretion was seen. In the continued presence of the cholinergic stimulus, secretion decreased by about 90% over a 4.5-h period (Fig. 1), although it was still enhanced relative to the secretion of protein from unstimulated glands at the same time (4.5 h).

While this decay or fall-off in protein secretion might have resulted solely from fatigue in the system, for example

decreased responsiveness to the stimulus or energy limitations relative to transport, it might also have been due to the diversion of digestive enzyme itself, which would remove the supply of available enzyme from a presumptive enteropancreatic circulation. To distinguish between these two possibilities, we collected pancreatic digestive enzyme secretion, stimulated as described above, measured its protein content, and immediately reintroduced it into the duodenum (without any modification of the material), thereby maintaining the continuity of the natural process. Under these conditions the fall-off was indeed less than during diversion (Fig. 1) (log protein output against time for the data in Figure 1; $b_{\text{diverted}} = -0.004$ ($r = 0.97$), $b_{\text{instilled}} = -0.0028$ ($r = 0.94$); b_{div} against b_{inst} , $P < 0.025$). A relatively steady protein output was maintained under these conditions for about 3 h, although not at maximal rates. The increase in protein secretion after protein instillation into the intestinal lumen was equal to $61.3\% \pm 10.6$ s.e.m. ($n = 6$) of the amount instilled for the complete experiment (relative to time-paired controls (diverted)), and reached a maximum of 83% for the period of peak response (Table 1). This increase in pancreatic protein output might have come about indirectly from the release of hormones into the blood after the instillation of pancreatic juice into the intestine as well as from the direct circulation of enzyme molecules. To test for this possibility, at least for the hormone known to produce substantial changes in total protein output by this gland, cholecystokinin-pancreozymin (CCK-PZ), an experiment was done in which pancreatic juice was again completely diverted, but at 120 min 1 Ivy

Table 1 Circulation of pancreatic enzymes under steady-state conditions

Condition	<i>a</i> Protein input at 210 min	<i>b</i> Protein output 210–240 min	<i>c</i> Control output 210–240 min	<i>b</i> – <i>c</i>	Percentage recirculated, [(<i>b</i> – <i>c</i>)/ <i>a</i>] × 100
Intestinal instillation	3.1	4.1	1.5	2.6	83%
Intravenous injection	3.6	4.4	1.5	2.9	80%

dog unit of purified CCK-PZ per kg body weight was injected in addition to the other stimulants (as in Fig. 1) for the remainder of the experiment. No significant additional augmentation of protein secretion was seen under these conditions.

By contrast, if the circulation of intact digestive enzyme did indeed account for the increased protein output, then the intravenous injection of pancreatic juice should produce a similar response to that seen after intestinal instillation. This was observed (Fig. 1). There was an increase in protein secretion relative to controls equal to $51.6\% \pm 5.5$ s.e.m. of the protein injected during the course of the complete experiment with a maximum of 80.2% for the period of peak response (Table 1) (log protein output against time for the data in Fig. 1; $b_{\text{injected}} = -0.0022$ ($r = 0.94$); b_{div} against b_{inj} $P < 0.001$).

The relationship between the amount of digestive enzyme instilled or injected and increased enzyme secretion as a result of either can be more clearly evaluated by plotting the response as a function of the amount of digestive enzyme instilled or injected in individual experiments. This is true because the amount of enzyme applied varied considerably from experiment to experiment. When the data were treated in this manner, the variance virtually disappeared and the response was proportional in a roughly linear fashion to input ($y = 0.92x + 3.75$, $r = 0.97$). The calculated slope was now 0.92 or the equivalent of 92% circulation. The difference between the overall responses (61% for instillation and 52% for injection) and the slope for the data as presented in Fig. 2 is found in the positive x -intercept ($x = 19.7$), which represents the amount of enzyme that must be added to the system before there is a response. This is the system 'capacitance' and may include various enzyme-containing compartments, such as the intestinal lumen, absorptive cells, blood, kidney, and of course the pancreas itself. Apparently, the size of the capacitance is roughly the same regardless of how the enzyme is administered since both injected and instilled experiments regressed similarly with approximately equal intercepts ($x_{\text{inst}} = 18$, $x_{\text{inj}} = 21.5$). The fact that one point was negative suggests that our function as calculated is slightly

displaced. If we correct this by setting the difference between control and experimental values equal to zero for the lowest response (the negative point) and adjust the other experimental points equivalently, the positive intercept of the x -axis is still substantial (uncorrected x -intercept = 19.7, corrected x -intercept = 13.5) and significantly different from 0 ($P < 0.001$).

While circulation of digestive enzyme is the most direct interpretation of these data, particularly since the potential for such a process has been demonstrated⁸ and also because input and output are almost equivalent at the steady state, the injected or instilled enzyme could still have produced the effect in other ways such as by the release of as yet undescribed secretagogues or by regulating enzyme secretion directly at the acinar cell level. If indeed the enzyme is being circulated, then, at least for the situation of maximally stimulated secretion in the absence of food, the efficiency of the process is remarkably high, of the order of 80–90%.

The conservation of digestive enzyme by its circulation could provide for a substantial saving of energy for digestion, that is, it would eliminate at least in part the need to metabolically recycle digestive enzyme by the liberation (protein breakdown), absorption, and reincorporation of amino acids into new protein.

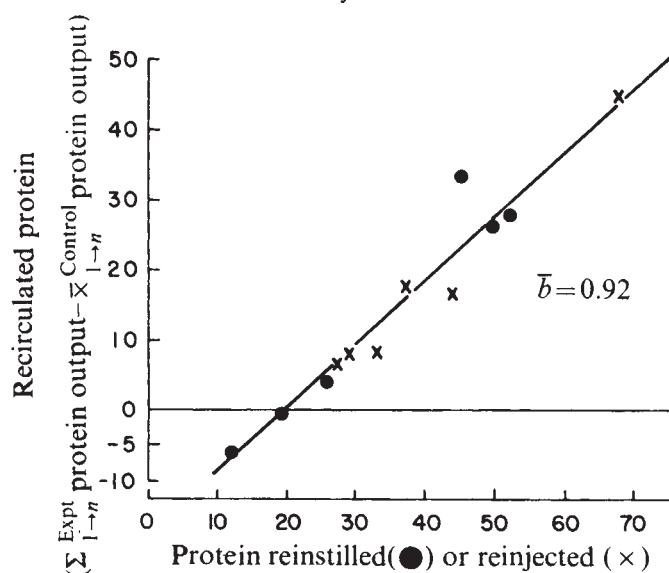
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Fig. 2 The relationship between enzyme input (intestinal instillation (●) or intravenous injection (×)) and the circulation of digestive enzyme in each individual experiment (the difference between total protein output in each experiment ($\Sigma_{i \rightarrow n}^{\text{Expt}}$) and the average total output for control experiments for the same duration ($\bar{X}_{1 \rightarrow n}^{\text{Control}}$). The amount of enzyme circulated varied directly and linearly with the amount of enzyme added to the system with a slope of 0.92 or 92% of the input being circulated. The positive x -intercept represents the input capacitance of the system.



Increased breakdown of glycosaminoglycans and appearance of corrective enzyme after skin transplants in Hunter syndrome

ATTEMPTS to treat the mucopolysaccharidoses with infusions of normal plasma or leukocytes have produced mixed results; some workers^{1–3} reported little or no positive effect, while others^{4–6} noted both clinical and biochemical changes after treatment. Among the more obvious reasons for these conflicting findings are differences in the responsiveness to treatment of genetically distinct forms of the disorders⁷, in the volumes of plasma administered and in the activities of the corrective factors in the plasma.

Replacement therapy by infusion of plasma can at best provide only a temporary solution, since the half lives of the enzymes involved are of the order of a few days only^{8–10}. The changes we observed immediately after infusion^{6,7,11,12} were, however, sufficient to encourage an attempt to provide a more permanent source of corrective factors. Accordingly, skin allografts were transplanted on a 4-yr-old boy (B.G.) with Hunter syndrome, who, 11 months earlier, had shown a positive response to plasma infusion therapy¹². The diagnosis was established first on the typical clinical features, second on probable X-linked inheritance, since the mother had another affected son by an earlier marriage and third, by cultured fibroblast correction experiments using typed reference cells. Both the mother (HL-A:

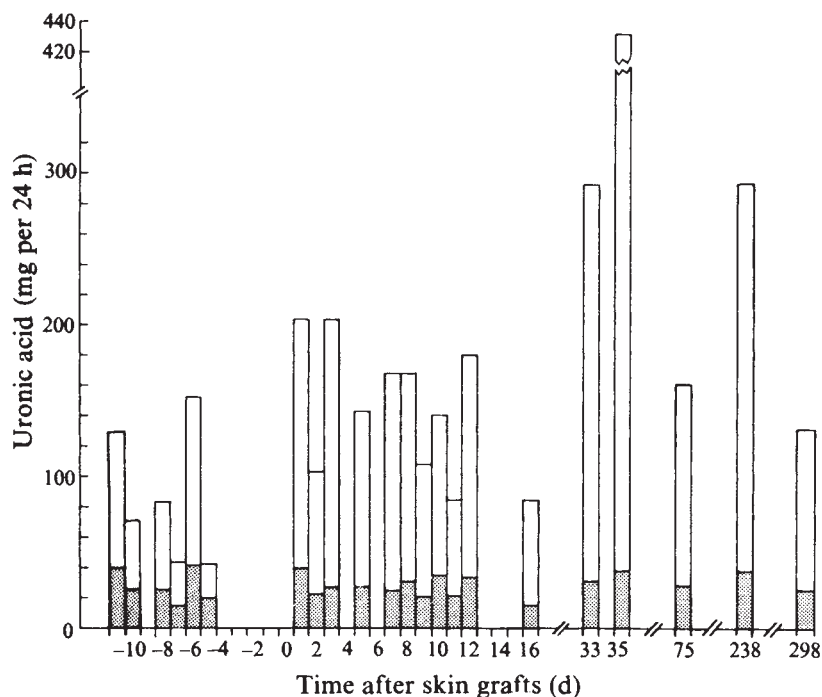


Fig. 1 Total 24-h excretion of urinary uronic acid before and after skin grafting. The day of grafting is designated day 0, and the days before grafting by negative numbers. Stippled areas of columns, polymeric uronic acid; open areas of columns, uronic acid fragments

W32,9,12,y) and the father (HL-A:1,2,8,17) had two HL-A antigens in common and two incompatible with the patient (HL-A: 1,9,8,y) (y indicates an unidentified antigen of the second series). In the mixed leukocyte reaction the patient's cells had no reaction when mixed with maternal cells but showed an approximate threefold increase in incorporated radioactivity when mixed with paternal leukocytes. But the patient received skin allografts from both parents, because it was reasoned that the dose of genes responsible for the Hunter corrective factor might be higher in skin cells derived from the father than from the mother (who was an obligatory heterozygote in whom an unknown proportion of wild genes on Lyonised X chromosomes might be inactive).

Full thickness elliptical skin 5 cm × 2 cm was taken from the inner side of the left upper arm of the mother and father, and grafted on the flexor aspect of the forearm of the child. Immunosuppressive treatment (azathioprine, 25 mg daily, prednisolone, 25 mg twice daily), was commenced 7 d before grafting and continued (at the same doses for 6 weeks and then on progressively smaller doses) for 9 months.

Initially both grafts were judged to have taken and were pink after 2 weeks. Four weeks after transplant the paternal graft was rejected. Six weeks after transplant it was judged that about 90% of the maternal graft had taken, but this too was rejected about 3 months after transplantation.

Urine collections (24 h) taken before and after grafting were stored at -20 °C with 10 ml of toluene until analysed. Polymeric glycosaminoglycans (GAG) were isolated from 200-ml samples by precipitation with 9-aminoacridine HCl and conversion to their sodium salts^{13,14} and the GAG fragments that remained in the supernatant solution were collected on a column of Dowex-1 × 2 (ref. 4) and eluted with 2 M sodium chloride. Both fractions were assayed for their uronic acid contents¹⁵. The GAG fragments eluted from Dowex were concentrated by positive pressure dialysis through a filter membrane (UM-05, Amicon) and 1-ml samples containing approximately 1 mg of uronic acid were chromatographed on a column (13 mm × 600 mm) of Sephadex G-25 'fine'. The relative proportions of high and low molecular weight fractions I and II respectively (Fig. 2) were determined by tracing the elution profiles on to thin paper, then cutting out and weighing the area under

each peak. Sulphate-uronic acid ratios were determined as before¹¹.

Crude Hunter corrective factor was isolated from urine samples using part of the method described by Cantz *et al.*⁸. The precipitate from urine made 70% saturated with ammonium sulphate was redissolved in water, insoluble residue was discarded after centrifugation, and the supernatant solution was made 50% saturated with ammonium sulphate. The resulting precipitate was removed by centrifugation and the supernatant solution was made 80% saturated. This last precipitate was again dissolved in water, freeze-dried and stored at -15 °C. Weighed amounts were redissolved in 1 ml of 0.01 M phosphate buffer, pH 6.0, in 0.15 M NaCl, dialysed against three changes of the same buffer (500 ml) at 2 °C for 20 h, then assayed for corrective activity⁸ using cultured fibroblasts from the patient.

Some 25–30% of the patient's total 24-h uronic acid was excreted as polymeric GAG before grafting (Fig. 1). After treatment the proportion of polymeric GAG decreased considerably, varying between 15 and 20% while at the same time total uronic acid excretion increased markedly, reaching a peak almost three times higher than pretreatment levels, on the 35th day after grafting. At this time polymeric GAG accounted for less than 10% of the total.

The GAG fragments eluted from Dowex were separated

Table 1 Changes in the size distribution and sulphate-uronic acid ratio of uronic acid oligosaccharides eluted from Sephadex G-25 before and after skin grafts

Day of treatment	Percentage of total uronic acid eluted		Sulphate-uronic acid ratio of Fraction II
	Fraction I	Fraction II	
- 12	48	52	1.7
- 9	48	52	—
- 5	56	44	—
+ 5	33	67	2.1
+ 11	40	60	—
+ 15	33	67	—
+ 33	26	74	—
+ 235	31	69	1.1
+ 296	16	84	1.2
+ 422	14	86	0.5

Fractions I and II are the high and low molecular weight components respectively shown in Fig. 2.

into three components by chromatography on Sephadex G-25 (Fig. 2), of which the largest was eluted closely behind the void volume followed by an oligosaccharide component of smaller molecular size and finally a tail fraction. Before treatment, the largest of these components contained approximately half the total uronic acid eluted from the column (Table 1), but by the fifth day after grafting this had decreased to one third of the total and the proportion continued to decrease with time, amounting to 26% by day 33 and 14% by day 422. Coupled with the increase in relative proportions of component II was a decrease in the ratio of SO_4 : uronic acid of this fraction from values near 2.0:1 down to only 0.5:1, 422 d after grafting.

Three normal children aged 4.5–8 yr excreted 89.2–104.7 U of Hunter corrective factor in their urine every 24 h. (1 U was defined by Cantz *et al.*⁸ as sufficient to induce half maximal correction of sulphate incorporation.) In contrast, the patient 270 d before skin transplant (and 42 d after receiving 1,600 ml of plasma from normal blood)

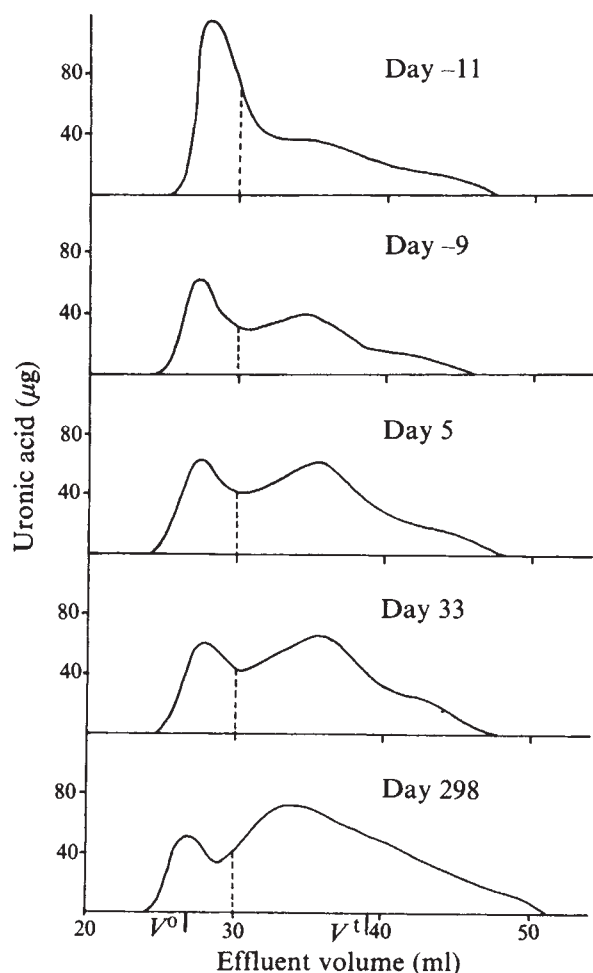


Fig. 2 Sephadex G-25 gel filtration profiles of uronic acid fragments eluted from Dowex-1. Samples containing 1 mg of uronic acid were eluted from the column in 0.2 M sodium acetate, pH 6.8. Fractions (0.85 ml) were collected and their uronic acid contents determined by an automated procedure. The dotted line indicates the boundary between fractions I and II (see Table 1). Sephadex filtration analysis was carried out on each of the six preoperative samples represented in Fig. 1. The two which are shown were selected because they show the extremes of preoperative variation. Thus the profile on day -9 had the greatest oligosaccharide component of any of the preoperative samples.

Table 2 24 h Urinary excretion of Hunter corrective factor

Source of urine	*Units of correction excreted per 24 h
Female control, age 4 yr 6 months	89.2
Male control, age 8 yr	104.7
Male control, age 4 yr 6 months	95.2
Patient, day -270†	0.86‡
Patient, day 15	0.00
Patient, day 54	0.12
Patient, day 98	3.62
Patient, day 237	4.89

*Units of correction were calculated from a dose-response curve constructed by plotting the degree of correction of the patient's fibroblasts (fall in radiolysulphate incorporation per mg of fibroblast protein) induced by serial dilutions of corrective factor⁸. Duplicate values agreed within $\pm 2.4\%$ except those for the female control which agreed to within $\pm 6.1\%$. Samples prepared from control urines and from the patient gave a mean corrective value of 0.34 units per 24 h after they had been boiled for 5 min. This control level was subtracted from all results obtained with unboiled preparations.

†Day 0 is defined as the day on which skin transplant was performed.

‡This urinary collection started 42 d after the patient had received an intravenous infusion of 1,600 ml of plasma from normal blood.

excreted only 0.86 U. As Table 2 shows, corrective factor excretion was low until 54 d after transplant but had risen significantly 98 and 237 d after transplant.

The large increase in excretion of uronic acid and in the relative proportions of oligosaccharides of low molecular weight, strongly suggest that in this patient, the grafts had released sufficient Hunter corrective factor to induce these changes. Since the defective enzyme in the Hunter syndrome is sulpho-L-iduronate sulphatase¹⁶, the increased desulphation of low molecular weight GAG further supports this view. We have reported similar evidence for GAG degradation after infusion of normal plasma^{6,7,11,12}, but on this occasion nonspecific effects attributable to an increase in blood volume after transfusion could be discounted; moreover the production of the Hunter corrective factor was confirmed by demonstrating its appearance in the urine.

The amount of corrective factor excreted increased during the period of observation, even though both grafts seemed clinically to have been rejected by day 100. It is possible, however, that some of the donor cells survived longer, since the amount of corrective factor excreted was still greatly in excess of pretreatment levels 237 d after grafting (Table 2), although its half life *in vitro* is only 2 d (ref. 8). Active concentration by fibroblasts of deficient enzymes has previously been demonstrated *in vitro* for other types of mucopolysaccharidosis^{9,10}. Thus, the delayed excretion of Hunter corrective factor observed in this case can be explained if all of the enzyme produced by the graft initially was taken up by the patient's cells. Alternatively the data are also consistent with the explanation that cells in the graft produced a factor which stimulated the patient's cells to produce corrective factor.

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Human serum lyses RNA tumour viruses

ALTHOUGH there is agreement that oncornaviruses infect and are associated with malignancies in mice, cats and chickens¹, the evidence for oncornavirus infection in man remains controversial². Similarly, antibodies to oncornavirus antigens have been detected in laboratory animals³⁻⁷, but there are few data indicating the presence of such antibodies in humans^{3,8,9}. These findings suggest that man possesses a natural defence mechanism which inhibits or interferes with oncornavirus infection and replication. We report here evidence for such a mechanism.

Oncornaviruses isolated from several animal species were found to be inactivated by fresh human serum. As determined by an XC cell plaque reduction assay¹⁰, a 1:2 dilution of human serum inactivated 2.5×10^5 plaque-forming units (PFU) of Moloney leukaemia virus (MLV). The 50% end-point dilution for all of five human sera tested was approximately 1:16 for AKR leukaemia virus, Friend leukaemia virus, MLV, and Rauscher leukaemia virus (Table 1). Similarly, by using a focus reduction assay, a 1:16 dilution of human serum inactivated Moloney sarcoma virus (MSV), simian sarcoma virus, and MSV-gibbon ape leukaemia virus pseudotype. There was, however, no demonstrable inactivation of oncornaviruses by heated (56 °C, 30 min) human serum or by fresh sera from guinea pigs, rabbits, or mice (BALB/c and NIH Swiss). To determine the mechanisms of viral inactivation, SCRF 179 cells, an established murine lymphoid line free of mycoplasma and persistently infected with MLV (SCRF 179 (MLV)), were grown in medium con-

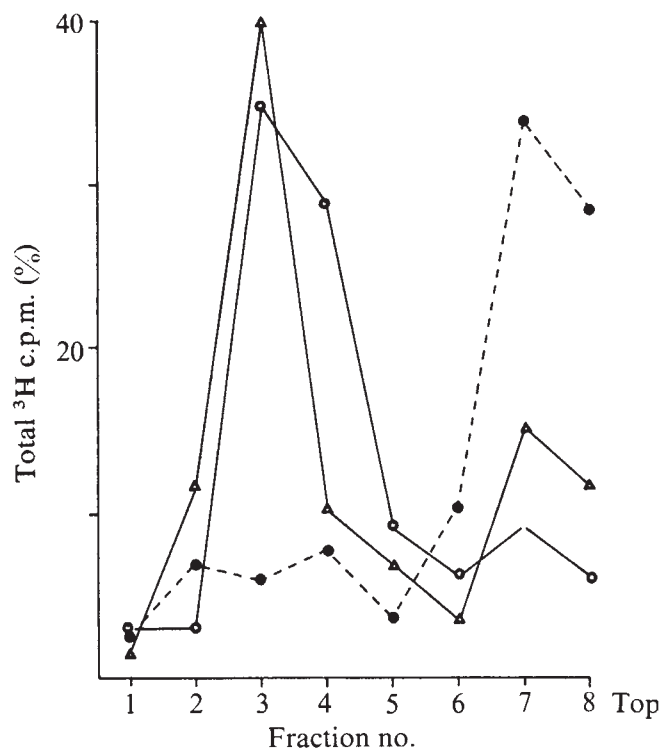


Fig. 1 Effect of human serum on sedimentation of MLV. MLV, labelled with ³H-uridine, was purified in sucrose gradients and, without a freeze-thaw, was reacted at 37 °C for 30 min with fresh human serum (●), heated (56 °C, 30 min) human serum (○), or 1% bovine serum albumin (△). The mixtures were layered on to 4.5 ml 20 to 50% (w/w) sucrose gradients in TES buffer (0.01 M Tris, pH 7.4; 0.05 M NaCl; 0.001 M EDTA). The gradients were centrifuged in a Spinco rotor SW 50.1 at 50,000 r.p.m. for 1 h and fractionated into 0.7-ml samples. Counts were made in Aquasol (New England Nuclear) using 20-μl samples.

taining ³H-uridine (15 μCi ml⁻¹; 40-50 Ci mmol⁻¹). Labelled MLV was purified, incubated for 30 min at 37 °C with bovine serum albumin (BSA), heated human serum or fresh human serum, and analysed by sucrose gradient ultracentrifugation. Figure 1 shows that after being incubated with either BSA or heated human serum the virus-associated radioactive counts sedimented to the lower portion of the gradient. This radioactivity was precipitable by 10% cold trichloroacetic acid (TCA) and resisted degradation by RNase (20 μg ml⁻¹). In contrast, when MLV was mixed with fresh human serum, most of the label remained at the top portion of the gradient and was not quantitatively precipitated by TCA. These results indicated that fresh human serum released RNA from the virus, presumably by lysis¹¹.

Fresh but not heated human serum released RNA-dependent DNA polymerase (RDDP) from six different oncornaviruses (Table 2) and such RDDP release could be

Table 1 Inactivation of oncornaviruses by human serum

Virus	Untreated	Plaque number after treatment:					With heated serum 1:2
		1:2	1:4	1:8	1:16	1:32	
AKR leukaemia virus	185	0	0	0	42	112	190
Friend leukaemia virus	206	0	0	0	48	127	210
Rauscher leukaemia virus	489	ND	0	14	69	370	498
Moloney leukaemia virus	880*	4	18	49	139	620	811

Equal volumes of virus and human serum (fresh or heated at 56 °C for 30 min) dilutions were incubated at 37 °C for 30 min. Duplicate 0.4-ml samples of the virus-serum mixtures were inoculated on to monolayers of secondary NIH Swiss mouse fibroblasts (24 h old) in the presence of polybrene (2 μg ml⁻¹). Cultures were refed 1 and 4 d after infection, irradiated with ultraviolet light, and overlaid with 10⁶ XC cells per Petri dish¹⁰. These cells were refed 1 d and fixed and stained 3 d after irradiation.

*Represents 88 plaques of a 1/10 dilution of input virus multiplied by 10.

Table 2 Release of oncornavirus RDDP by human serum

Enzyme source	Virus source	Serum type	c.p.m. polymerised ³ H-TTP	
			Fresh serum	Heated serum
None-H ₂ O	—	—	327	376
Purified RDDP*	—	Normal	1,070	943
Avian myeloblastosis virus	Chicken serum	Normal	728	350
Feline leukaemia virus	NCI	Normal	6,280	1,169
Feline leukaemia virus	NCI	C4 depleted	849	842
Moloney leukaemia virus	YCAB cells	Normal	2,153	466
Moloney leukaemia virus	YCAB cells	C2 deficient	587	587
Moloney leukaemia virus	SCRF 179 cells	Normal	3,064	393
Moloney leukaemia virus	SCRF 179 cells	C4 depleted	495	474
Rauscher leukaemia virus	NCI	Normal	2,383	897
Rauscher leukaemia virus	NCI	C2 deficient	730	696
Simian sarcoma virus	NCI	Normal	708	452
Wild mouse virus 1504	Wild mouse 1504 embryo cells	Normal	1,055	343

Enzyme source (25 μ l) was incubated with 25 μ l fresh or heated (56 °C, 30 min) human serum for 30 min at 37 °C. After incubation an equal volume of RDDP assay mix was added to the RDDP-serum solution, and the samples were again incubated at 37 °C for 30 min. The assay mix contained 1.25 mM MnCl₂; 0.05 M Tris buffer, pH 8.2; 20 mM dithiothreitol; 100 μ M deoxyadenosine 5'-triphosphate; 100 μ M deoxycytidine 5'-triphosphate; 100 μ M 2-deoxyguanosine 5'-triphosphate; 10 μ M thymidine 5'-triphosphate; poly(rA)-oligo(dT)₁₂₋₁₈ (0.33 U ml⁻¹) and thymidine-methyl-³H 5'-triphosphate (100 μ Ci ml⁻¹, specific activity 18 Ci mmol⁻¹). Polymerised ³H-TTP was counted in Aquasol after collection on DEAE filters and subsequent washes with 5% Na₂HPO₄, water, ethanol and ether¹². The degree of RDDP release caused by serum (which influences the RDDP assay) compared with NP40 varied greatly with each virus preparation and depended on virus concentration and amount of inactivated virus. For instance, this preparation of feline leukaemia virus released 108,000 c.p.m. of enzyme activity after NP40 treatment, whereas the simian sarcoma virus preparation released only 8,200 c.p.m. after NP40 treatment.

*Purified RDDP was from MLV.

detected at serum dilutions up to 1:8. Since RDDP is located within the virion, the release of this enzyme is a measurement of viral lysis. All normal sera from 32 separate humans tested but none from six individual guinea pigs, four individual rabbits, or pools from two separate strains of mice (BALB/c, NIH Swiss) caused the release of RDDP. The tested normal human sera were from five different cord blood samples and from six leukaemic and 21 healthy adults of both sexes with functional complement (C) activity.

Table 2 also shows that human sera genetically deficient in C2 or immunochemically depleted of C4 failed to release RDDP. These observations, coupled with the finding that all tested fresh normal sera released RDDP, suggested that C was required for the viral lysis. Consistent with this interpretation is the finding of C activation by MLV. After mixture of 200 μ l of fresh human serum with 200 μ g of purified MLV (in 200 μ l) for 60 min at 37 °C, 89% of the total complement haemolytic 50% endpoint (CH₅₀) units were consumed. Comparable C consumption was also observed when the reaction of serum with MLV was carried out in the presence of RNase (2.3 mg ml⁻¹) and DNase (1.6 mg ml⁻¹) (final concentrations in the serum-virus mixture), indicating that viral or contaminating cellular RNA or DNA was not responsible for the observed consumption of C. Haemolytic C component titrations¹³ in serum incubated with virus revealed significant consumption of the terminal components of C, that is C8 (40% above background) and C9 (20% above background), which are required for membrane lysis in other systems^{13,14}. In addition, the conversion of C3 and C4 in the serum was demonstrated by immunoelectrophoresis.

We were unable to establish a role for antibody in this reaction after trying four methods. First, fluoresceinated monospecific antisera against human IgA, IgG or IgM failed to stain the surfaces of SCRF 179 (MLV) cells which had previously been incubated with human serum. These cells demonstrated cell surface MLV antigens detectable by immunofluorescence with rat antibodies to MLV. Second, human serum heated at 56 °C for 30 min failed to inhibit oncornavirus plaque formation or to release RDDP either alone or in conjunction with fresh guinea pig or rabbit sera. Third, sera from two agammaglobulinaemic patients released RDDP from oncornavirions. These sera had less than 10 μ g IgG per ml and no detectable IgA or IgM. Fourth, fresh human serum retained its ability to release RDDP from oncornaviruses (MLV and feline leukaemia

virus) after absorption with SCRF 179 (MLV) cells. (This serum was absorbed twice with 1×10^7 cells for 40 min at 4 °C in the presence of 0.01 M EDTA. Before use, the EDTA in the serum was neutralised with CaCl₂.)

To rule out the possibility that lysis of oncornaviruses occurred because of C activation by components of foetal calf serum retained on the viral surface, we purified MLV from SCRF 179 (MLV) cells grown in medium containing heat-inactivated human serum in place of foetal calf serum. When mixed 1:2 with fresh human serum, these virions released significant amounts of RDDP and did not form plaques on XC cells (control without serum-3180 PFU, heated serum-2880 PFU, fresh serum-0 PFU). Avian myeloblastosis virus purified directly from chicken serum also released RDDP on incubation with fresh human serum (Table 2), and MLV obtained directly from a 1:10 dilution of mouse plasma (14,500 PFU) was inactivated by a 1:2 dilution of fresh human serum (heated human serum—confluent (>5,000) PFU, fresh human serum—263 PFU). Fresh guinea pig serum did not reduce plaque formation by this same MLV preparation from mouse plasma.

Our results demonstrate that all tested human sera with functional C sources inactivate and lyse avian, feline, murine and simian oncornaviruses. Whether a similar mechanism inactivates other enveloped viruses is unknown and is under study in our laboratory. C seems to be a mediator of this lytic mechanism, since human sera deficient in C2 or C4 do not release RDDP from oncornaviruses. In contrast, we found no evidence for antibody involvement in this reaction. Not only did agammaglobulinaemic human sera lyse oncornaviruses, but absorption of normal human serum with cells expressing surface oncornavirus antigens also failed to remove its cytolytic potential, and heated human serum was unable to sensitise oncornaviruses for lysis with guinea pig C. These findings suggest either that trace amounts of heat labile antibody are present or that human C but not guinea pig, rabbit or mouse C can be directly activated by oncornaviruses. Similar activation of C in the absence of antibody has been reported in other systems^{15,16}. Whether serum factors other than C or antibody are involved is not known. If oncornaviruses do infect man, lysis of these viruses may represent a natural resistance mechanism which would limit horizontal oncornavirus transmission and serve to maintain vertically transmitted oncornaviruses in a repressed, defective or latent state. It would be interesting to examine the status of the C system

in individuals with neoplasia and to determine the incidence of malignancies and oncornavirus expression in humans with deficiencies in the C system.

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Presence of antibody to a primate RNA virus in human plasma

AN antigen is present in leukocytes of patients with chronic myelogenous leukaemia (CML) which cross reacts with an antigen of the core protein of an RNA virus originally isolated from a Rhesus monkey, the Mason-Pfizer monkey virus (MPMV). This finding depended on development of a radioimmune assay using rabbit antibody raised against MPMV¹. I report here that using a modified Farr technique² plasma from some patients with various malignancies has antibody which reacts the MPMV core protein. The inference is that individuals in the human population may have been infected with a virus which is identical with or has antigens similar to those of the MPMV virus. The possible relevance of such infections to development of malignancies has yet to be determined.

A major protein of MPMV having a molecular weight of 25,000 (p25) was purified by a combination of Agarose gel filtration and DEAE-cellulose column chromatography. For a typical purification experiment, 10 ml of concentrated MPMV (5×10^{10} - 10^{11} particles ml⁻¹) grown in NC-37 cells were disrupted by exposure to 1% NP40 at 4°C for 2-3 h. The mixture was separated on an Agarose A 0.5 M (BioRad) column (1.5 cm × 90 cm) equilibrated with 0.1% NP-40 in TNE (0.01 M Tris-HCl (pH 8.3), 0.15 M NaCl, 0.002 M ethylenediamine tetra-acetate) buffer. The peak containing p25 was applied to a DEAE-cellulose column (1.5 cm × 15 cm) equilibrated with 0.02 M phosphate buffer (pH 7.5) and eluted with 0.15 M sodium chloride. The isolated p25 was more than 95% pure as estimated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. The p25 was iodinated with ¹²⁵I by the chloramine T procedure³. A goat anti-rabbit antiserum gave a maximum of 72% indirect precipitation of a mixture of ¹²⁵I-p25 and a rabbit antiserum prepared against MPMV. This indirect radio-precipitation was inhibited by MPMV virus obtained from

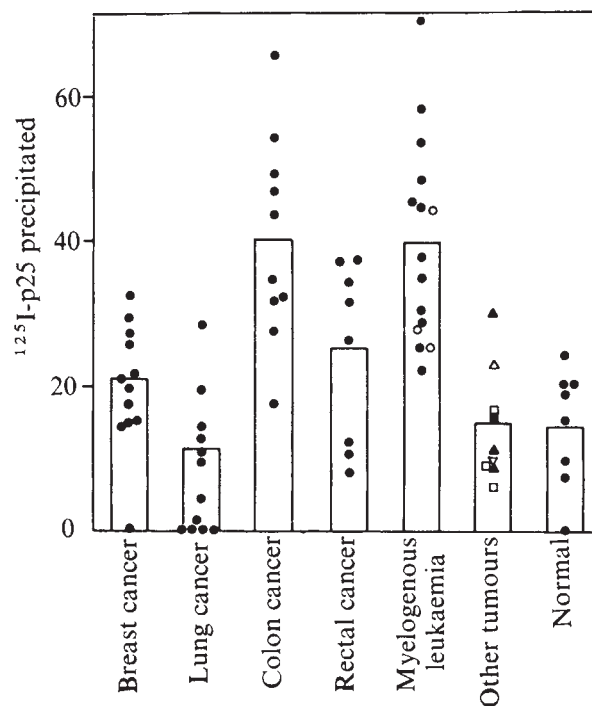


Fig. 1 The levels of antibody in each plasma are represented by the symbols. The individuals studied have been arbitrarily grouped in broad categories as indicated; the height of the bar represents the mean for each group. Myelogenous leukaemia group: ●, AML; ○, CML. Other tumours: ■, melanoma; □, osteosarcoma; ▲, carcinoma of larynx; ▽, acute lymphatic leukaemia; △, liposarcoma. Aliquots of heparinised plasma (200 µl) were incubated with ¹²⁵I-MPMV p25 and precipitated with 14% Na₂SO₄ as described in the legend of Table 1. The amount of ¹²⁵I-p25 precipitated by a plasma was converted to the percentage of ¹²⁵I-p25 precipitated assuming that the rabbit anti-MPMV precipitated 100% and the rabbit anti-MMTV precipitated 0%.

either cultures of human (NC-37) or simian (MT) cells infected with the virus. Proteins obtained from viral density region of disrupted non-infected NC-37 or simian cells (that is, in the region of 1.15-1.19 g ml⁻¹ in a sucrose density gradient) did not, however, cause any detectable inhibition of precipitation. Apparently then, the p25 is a viral protein and not a membrane component of the host cell, a finding in agreement with previous reports⁴.

In preliminary observations the conditions for an assay to be used for a survey of human plasmas were set. The percentage of ¹²⁵I-p25 precipitated with 14% sodium sul-

Table 1 ¹²⁵I-MPMV p25 bound to 14% sodium sulphate precipitates of human plasma and control antisera

Plasma	% bound to 14% Na ₂ SO ₄ precipitates of plasma			
	1:16	1:8	1:4	1:2
AML*	24.0	35.8	46.4	54.3
Normal individuals	12.4	13.4	15.5	16.8
Rabbit anti-MPMV	68.0	71.6	71.4	74.3
Rabbit anti-MMTV	NT	NT	12.8	13.2

*AML, Acute myelogenous leukaemia. NT, not tested.

The volume of each sample was adjusted to 400 µl with 0.1% BSA in TNE buffer. ¹²⁵I-MPMV p25, 2-3000 c.p.m. of specific activity of 5×10^6 c.p.m. µg⁻¹ was added to the sample and incubated 1 h at 37°C. Final concentration of 14% Na₂SO₄ was made with 25% Na₂SO₄ solution followed by an additional 1 h incubation at 37°C. Two-thirds of the supernatants (sup) were counted separately from the precipitates (ppt) with remaining supernatants. Then the percentage precipitation was calculated: % ppt = 100 [(ppt + 1/3 sup) - 1/2 (2/3 sup)] / [(ppt + 1/3 sup) + (2/3 sup)]. Aggregation of ¹²⁵I-p25 was minimised by storing in TNE buffer containing 10% glycerol and 1% Triton X-100. ¹²⁵I was prepared fresh every third week for the assays. In these conditions less than 4% of p25 was precipitable by 14% Na₂SO₄ without added plasma.

Table 2 Specific bindings of ^{125}I -MPMV p25 to purified human or rabbit IgG-coupled Sepharose columns

	Bindings of ^{125}I -P25 to IgG-Sepharose columns (c.p.m.)			Bindings of ^{125}I -BSA to IgG-Sepharose columns (c.p.m.)		
	RMTV	LHP	RMPV	RMTV	LHP	RMPV
Bound after wash	1,183	10,998	32,411	924	1,300	1,368
Elution by Glycine-HCl (pH 2.3)	210	2,895	13,342	182	309	296
Bound after Glycine-HCl (pH 2.3)	916	7,620	18,384	791	943	1,179
Elution by 6 M Guanidine-HCl	NT	2,087	10,921	NT	218	215
Bound after 6 M Guanidine-HCl	NT	6,500	8,775	NT	880	1,015
p25 Bound after wash	NT	NT	NT	NT	14,374	27,128

RMTV: rabbit anti-MMTV (mouse mammary tumour virus); LHP: plasma from a patient with acute myelogenous leukaemia; RMPV: rabbit anti-MPMV.

NT, not tested.

phate was measured using dilutions of plasma ranging from 1:2 to 1:16 (Table 1). Accurate quantitation of antibody present in large amounts in the rabbit anti-MPMV serum would require high dilutions of plasma. However, a plasma dilution of 1:2 would permit qualitative demonstration of antibody in plasmas containing either a great deal or only small amounts of antibody to ^{125}I -p25 as shown for two human sera (Table 1).

The binding of ^{125}I -p25 is at least partially due to IgG in the plasmas as shown in the following way. IgG was purified from various rabbit and human plasmas by successive 18% and 14% N_2SO_4 precipitations followed by DEAE-cellulose column chromatography. A single typical IgG precipitin arc was obtained for each preparation on immunoelectrophoresis using appropriate goat anti-human or anti-rabbit serum. Forty milligrams of each purified Ig was coupled to 4 ml of CNBr activated Sepharose. For each immunoabsorbent column, 1 ml of Sepharose was used. Radiolabelled antigens (^{125}I -p25, 51,000 c.p.m.; ^{125}I -BSA, 57,000 c.p.m.) were added to the column and incubated for 2 h at room temperature. The Sepharose columns were washed with $\times 20$ volumes of 0.2% ovalbumin in TNE buffer to remove unbound radiolabelled antigens. Successive elutions were made with 5 ml of 0.1 M glycine-HCl buffer (pH 2.3) and 6 M guanidine-HCl. As shown in Table 2, the purified Ig which was obtained from whole plasma bound ^{125}I -p25 specifically.

The survey of plasmas for antibody against ^{125}I -p25 was done on samples from 75 individuals, 67 of whom had a clinical diagnosis of malignancy and eight of whom were apparently healthy volunteers including three normal pregnant women. The samples were obtained from patients at Billings Hospital, University of Chicago and Presbyterian Hospital, Columbia University, New York. These donors were selected without regard to age, sex, stage or type of the malignancy or therapy.

The percentage of ^{125}I -p25 bound by each plasma is plotted in Fig. 1. The individual responses for 8 normal volunteers, 8 patients with carcinoma of the rectum, 12 patients with carcinoma of the lung, 13 patients with carcinoma of the breast and 9 patients with other malignancies range from 15 to 25% binding of ^{125}I -p25; differences between the means of these groups are not statistically significant ($P > 0.05$). Patients with carcinoma of the colon and leukaemia, however, had levels as high as 66–70% binding; furthermore, plasmas from only single individuals in each of these groups have less antibody than the normal individual with the highest level of binding. The significance levels of differences between the means are: $P < 0.005$ for patients with carcinoma of the colon and the normal group and $P < 0.001$ for patients with leukaemia and the normal group.

These results suggest that some individuals may have

been or are infected with a virus similar to or identical with MPMV. The demonstration of an antigen in leukocytes of patients with CML¹ is consistent with this possibility. In so far as it has been examined, antigens cross reactive with p25 of MPMV have not been found in other C type RNA viruses, but such cross reactions might be found on more extensive survey using highly sensitive radioimmune assays.

Patients with leukaemia or carcinoma of the colon may simply be more prone to exposure and/or infection with the agent responsible for inducing antibody against p25 of MPMV. Alternatively, of course, the infection may be relevant to development of malignancy. MPMV-like particles have been identified in human cell lines derived from a leukaemia or other malignancies^{2–7}, as well as from spontaneously transformed normal cells^{8,9}. The MPMV has morphological, biochemical and biophysical characteristics of known oncornaviruses^{10,11} though they have not been shown to share immunologic characteristics^{4,8}. Considerable evidence now supports the general proposition that viruses have aetiological significance in leukaemogenesis in humans¹² as well as in mice¹³. In mice, humoral antibodies to endogenous or exogenous leukaemia viruses presumably reflect exposure to the virus^{14,15}; the demonstration here of antibody to p25 of MPMV may have analogous significance. This possibility might be best studied in humans with CML, since leukocytes from such patients contain a protein antigenically cross reactive with p25 of MPMV and plasma from many such patients contains considerable antibody to the antigen. Furthermore, long remissions of the disease often occur. Therefore, in studies under way, changes which may occur in levels of the leukocyte antigen and in plasma levels of antibody will be studied during the course of the disease.

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Discrimination between eukaryotic and prokaryotic, and formylated and non-formylated, initiator tRNAs by eukaryotic initiation factor EIF-3

PROKARYOTIC and eukaryotic cells contain two methionine accepting species of transfer RNA, $tRNA_{Met}^{Met}$ and $tRNA_{Met}^{Met}$ (prokaryotes⁴ and non-formylated Met- $tRNA_i$) in prokaryotes⁴ and non-formylated Met- $tRNA_i$ from eukaryotes⁵⁻⁹ act exclusively in chain initiation; Met- $tRNA_m$ provides amino acids for internal positions in proteins⁴⁻⁹. Thus the structure of $tRNA_{Met}^{Met}$ is likely to have some unique attribute that makes it specific for initiation and that provides for its recognition by initiation factors¹⁰⁻¹³. We have investigated the specificity of an eukaryotic initiation factor (EIF-3) for initiator tRNA. A preparation of EIF-3 formed a complex preferentially with nonformylated eukaryotic Met- $tRNA_i$: the factor discriminated against the formylated eukaryotic species and against prokaryotic initiator tRNA whether it was formylated or not. A preliminary account of the experiments has been reported before¹⁴.

We have isolated EIF-3 from mouse Krebs II ascites cells by chromatography of a 0.25 M KCl ribosomal salt wash on DEAE-Sephadex and hydroxylapatite (details of the isolation of EIF-3 will be presented elsewhere). EIF-3, like initiation factor IF-2 from *E. coli*¹¹⁻¹², forms a ternary complex with GTP and eukaryotic Met- $tRNA_i$; the ternary complex is quantitatively retained on Millipore filters. The factor does not form a ternary complex with Met- $tRNA_m$ or other tRNA species. A similar factor has been prepared from rabbit reticulocytes and L cells¹⁵⁻¹⁸. The preformed ternary complex is transferred to 40S ribosomal subunits in the absence of mRNA. The nature of the partial reactions catalysed by EIF-3 (that is the formation of the ternary complex and its transfer to 40S ribosomal subunits) suggests that the factor is involved in the recognition of Met- $tRNA_i$, and is therefore an intermediate in the formation of a 40S preinitiation complex.

We compared the recognition by EIF-3 of eukaryotic and prokaryotic Met- $tRNA_i$ and fMet- $tRNA_i$. EIF-3 formed a ternary complex with GTP and yeast or ascites cell Met- $tRNA_i$ very rapidly (Fig. 1); however, there was little (only about 15% as much) ternary complex formation when *E. coli* Met- $tRNA_i$ was used (Fig. 1). Formylated prokaryotic Met- $tRNA_i$ was an even poorer substrate for EIF-3 (Fig. 1). Similar results were obtained when complex formation was measured as a function of the amount of EIF-3 (Fig. 2).

We next investigated whether EIF-3 distinguished between formylated and non-formylated Met- $tRNA_i$ (Table 1). Although EIF-3 clearly used non-formylated eukaryotic (yeast or ascites cell) Met- $tRNA_i$ preparations more efficiently than the formylated variety, it appeared that the factor would form a complex with the formylated species, albeit only 35-50% as well (Table 1). The formylation reaction was, however, only 65-80% effective; thus complex formation could have been with contaminating non-formylated Met- $tRNA_i$. Indeed, when a preparation of ascites cell fMet- $tRNA_i$ that contained 9,400 c.p.m. and was 64% formylated was incubated with GTP and increasing amounts (up to 20 μ g) of EIF-3, the radioactivity in the ternary complex, when a plateau was reached, was 4,300 c.p.m.; binding was maximum with less than 10 μ g of EIF-3

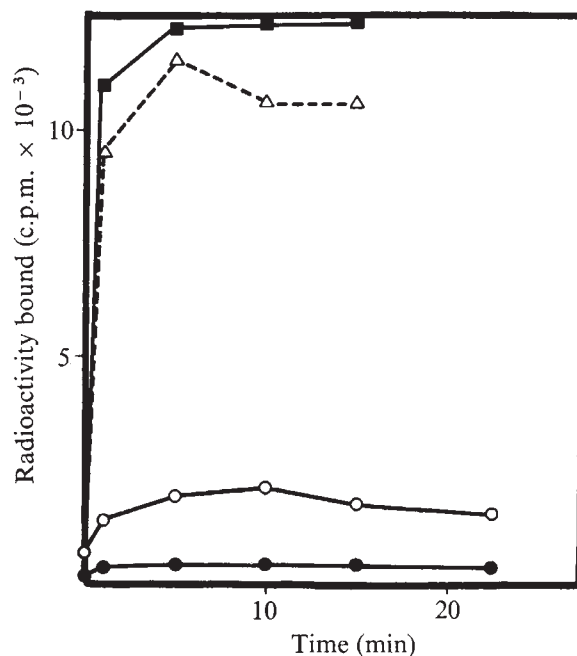


Fig. 1 Kinetics of ternary complex formation with EIF-3, GTP and eukaryotic or prokaryotic initiator tRNA. The reaction mixture for the eukaryotic initiator tRNA experiments contained in 100 μ l of buffer A (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.2 mM magnesium acetate, and 1 mM dithiothreitol): 34.2 μ g of EIF-3; 0.2 mM GTP; and 200 μ g of ascites cell tRNA containing 76,000 c.p.m. of 3H -Met- $tRNA_i$, or 125 μ g of yeast tRNA containing 65,000 c.p.m. of 3H -Met- $tRNA_i$. For the prokaryotic initiator tRNA experiments, 150 μ l of buffer A contained: 51.3 μ g of EIF-3; 0.2 mM GTP, and 19 μ g of *E. coli* $tRNA_{Met}^{Met}$ with 66,600 c.p.m. of 3H -Met- $tRNA_i$ or 17.3 μ g of *E. coli* $tRNA_{Met}^{Met}$ with 74,000 c.p.m. of formyl- 3H -Met- $tRNA_i$. Samples were incubated at 30 °C; at intervals, 25- μ l samples were removed and the formation of a ternary complex determined by filtration on Millipore filters. The filters were washed three times with 3 ml of buffer A. The filters were dried and placed in glass vials containing 5 ml of scintillation fluid¹⁹. Radioactivity was determined in a Packard Tri-Carb spectrometer with an efficiency of 16%. The $tRNA_{Met}^{Met}$ species was prepared from unfractionated ascites cell tRNA or from yeast tRNA by chromatography on BD-cellulose⁵. The purified *E. coli* $tRNA_{Met}^{Met}$ was obtained from Oak Ridge National Laboratory. The tRNA was charged with 3H -methionine (4.3 Ci mmol⁻¹) using a preparation of *E. coli* aminoacyl-tRNA synthetases^{3,20}. When 3H -Met- $tRNA_i$ was to be formylated, tetrahydrofolic acid (Leucovorin, Lederle Laboratories) was included in the reaction mixture²¹ and the extent of formylation was determined by paper chromatography of a deacylated sample²¹. ■, Yeast Met- $tRNA_i$; △, ascites Met- $tRNA_i$; ○, *E. coli* Met- $tRNA_i$; ●, *E. coli* fMet- $tRNA_i$.

(results not shown). The results are by no means conclusive, but since EIF-3 prefers the non-formylated species they are consistent with complex formation having occurred predominantly, if not exclusively, with the non-formylated Met- $tRNA_i$ that was present.

We sought to confirm our impression that it was chiefly non-formylated Met- $tRNA_i$ contaminating preparations of fMet- $tRNA_i$ that was responsible for ternary complex formation (Table 2). The complex formed with EIF-3, GTP and partially (64%) formylated Met- $tRNA_i$ was isolated on Millipore filters and digested with pancreatic RNase. The amount of Met- $tRNA_i$, as methionyl-adenosine and fMet- $tRNA_i$ as formyl-methionyl-adenosine²², in the RNase digest was determined after extraction with ethyl acetate²³. Only about 14% of the ternary complex contained fMet- $tRNA_i$. The predominant species in the complex (85-90%) was Met- $tRNA_i$. Cashion and Stanley²⁴ have reported similar results: they found a like amount (10%) of ternary complex formation when formylated eukaryotic Met- $tRNA_i$, rather than the non-formylated species, was tested with an initiation factor (IF₁) from rabbit reticulocytes.

Table 1 Discrimination by EIF-3 between eukaryotic and prokaryotic initiator tRNA

Initiator tRNA	Aminoacyl-tRNA bound (pmol)
Met-tRNA _f (ascites)	3.53
fMet-tRNA _f (ascites) (70% formylated)	1.28
Met-tRNA _f (yeast)	3.55
fMet-tRNA _f (yeast) (80% formylated)	1.80
Met-tRNA _f (<i>E. coli</i>)	0.59
fMet-tRNA _f (<i>E. coli</i>) (99% formylated)	0.11

The reaction mixture (25 μ l in buffer A) contained: 5.7 μ g of EIF-3; 0.2 mM GTP; and 40 μ g of ascites cell tRNA containing 13,800 c.p.m. of ³H-Met-tRNA_f, or 84 μ g of ascites cell tRNA containing 10,400 c.p.m. of formyl-³H-Met-tRNA_f, or 25 μ g of yeast tRNA containing 13,000 c.p.m. of ³H-Met-tRNA_f, or 24 μ g of yeast tRNA containing 16,000 c.p.m. of formyl-³H-Met-tRNA_f, or 3.1 μ g of *E. coli* tRNA_f^{Met} containing 11,100 c.p.m. of ³H-Met-tRNA_f, or 2.9 μ g of *E. coli* tRNA_f^{Met} containing 12,400 c.p.m. of formyl-³H-Met-tRNA_f. Incubation was at 30 °C for 5 min and the complex was assayed on Millipore filters.

Once again there was little ternary complex formation with EIF-3, GTP and prokaryotic Met-tRNA_f; however, even that small amount was reduced if *E. coli* Met-tRNA_f was formylated (Table 1).

The eukaryotic initiation factor EIF-3 shows a clear preference for non-formylated eukaryotic Met-tRNA_f. Formylation decreases the ability of the eukaryotic initiator tRNA to serve as a substrate for EIF-3, and prokaryotic Met-tRNA_f, whether formylated or not, serves especially poorly in the reaction. The observations are consistent with the finding reported by Heckman *et al.* (J. Heckman, G. Temple, C.L. Woodley, N.K. Gupta and U.L. RajBhandary, personal communication) that prokaryotic Met-tRNA_f is much less efficient (20–30%) than eukaryotic Met-tRNA_f in the translation of haemoglobin mRNA in mouse Krebs II ascites extracts. It is likely that the results reflect differences in the structure of eukaryotic and prokaryotic initiator tRNAs, initiation factors, or both. There is no extensive homology in the nucleotide sequence of initiator tRNAs from *E. coli*²⁵ and eukaryotes^{26–28}, and the initiation factors are almost certainly different.

The efficient formation of a ternary complex between mouse ascites cell EIF-3 and Met-tRNA_f from yeast, a phylogenetically distant eukaryote, implies conservation of the recognition sites. Indeed, the results were to be expected since the nucleotide sequence of initiator tRNAs from higher eukaryotes (rabbit liver²⁷, sheep mammary gland²⁷ and mouse P3 myeloma²⁸) have extensive homology with yeast initiator tRNA²⁶.

Lodish *et al.*²⁹ had reported that yeast fMet-tRNA_f was

used as effectively as Met-tRNA_f in initiation of protein synthesis by rabbit reticulocyte ribosomes. On the other hand, other available results^{6,30,31} more nearly approximated ours: that the incorporation of formylmethionine from eukaryotic fMet-tRNA_f into polypeptides in mouse Krebs II ascites extracts was slow and never very extensive when compared with that with Met-tRNA_f. The conflict may reflect to some degree difference in the cells used, but it may be important that in the experiments of Lodish *et al.*²⁹ high concentrations of purified initiator tRNA were used, concentrations so high as to risk dampening the differences in efficiency of incorporation from Met-tRNA_f and fMet-tRNA_f. The observations emphasise the great importance of using physiologically proper substrates for studying the partial reactions in the initiation of protein synthesis.

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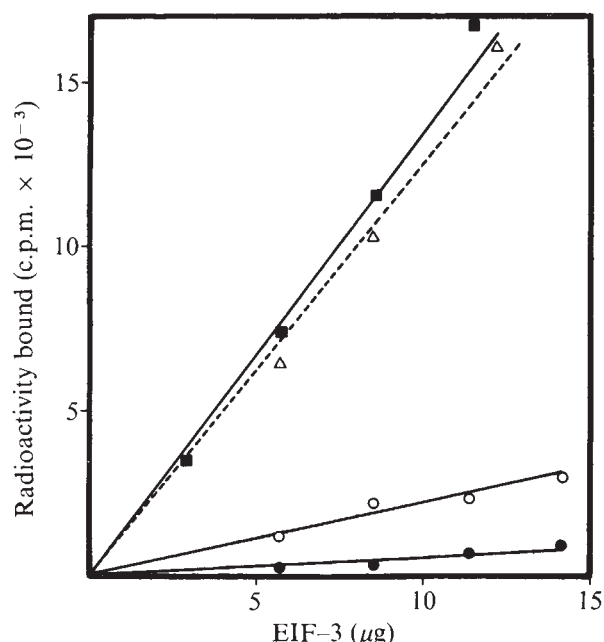


Fig. 2 Effect of the amount of EIF-3 on the formation of a ternary complex with eukaryotic or prokaryotic initiator tRNA. The reaction mixture (25 μ l in buffer A) contained: EIF-3 (as indicated); 0.2 mM GTP; and 100 μ g of ascites cell tRNA containing 25,000 c.p.m. of ³H-Met-tRNA_f, or 37.5 μ g of yeast tRNA containing 19,500 c.p.m. of ³H-Met-tRNA_f, or 2.9 μ g of *E. coli* tRNA_f^{Met} containing 12,400 c.p.m. of formyl-³H-Met-tRNA_f, or 3.1 μ g of *E. coli* tRNA_f^{Met} containing 11,000 c.p.m. of ³H-Met-tRNA_f. Incubations were at 30 °C for 5 min. Samples were assayed as described in Fig. 1. Symbols as in Fig. 1.

Table 2 Ternary complex formation with EIF-3 and fMet-tRNA_f or Met-tRNA_f

Sample	Met-tRNA _f or fMet-tRNA _f bound to Millipore filter (pmol)	Met-tRNA _f or fMet-tRNA _f remaining bound to Millipore filter after RNase digestion (pmol)	fMet-tRNA as fMet-adenosine in ethyl acetate phase (pmol)	Met-tRNA _f as Met-adenosine in aqueous phase (pmol)
Control	1.94	—	—	—
Digested with RNase and extracted with ethyl acetate	—	0.30	0.19 (13.7%)	1.20 (86.3%)

The reaction mixture (25 μ l in buffer A) contained: 7.6 μ g of EIF-3; 59.5 μ g of tRNA containing 12,200 c.p.m. of ³H-fMet-tRNA_f (64% formylated); and 0.2 mM GTP. Incubation was for 5 min at 30 °C. The EIF-3-GTP-Met-tRNA_f or EIF-3-GTP-fMet-tRNA_f complex was collected on Millipore filters. One group was used to determine the total amount of complex formed; the other was digested with (6 μ g) pancreatic RNase in 1 ml of 0.1 M potassium acetate buffer, pH 5.5 at 30 °C for 15 min. The complex remaining bound to Millipore filters was assayed (see Table). The fMet-tRNA_f as fMet-adenosine²² in potassium acetate buffer (pH 5.5) was extracted with ethyl acetate according to Leder and Bursztyn²³ and the Met-tRNA_f as Met-adenosine²³ remaining in the aqueous phase was determined. The values are the average of duplicate samples.

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DBAE-cellulose, a derivative with covalently bound dihydroxyboryl groups. ASV ^{32}P -labelled genome RNA was mixed with ^3H -methyl-labelled reovirus mRNA, digested to completion with RNase T_2 , and applied to a column of DBAE-cellulose. The 5'-terminal RNase T_2 fragment of reovirus mRNA, $\text{m}^7\text{G}(5')\text{ppp}(5')\text{G}^{\text{m}}\text{pCp}$, contains all the methyl groups of the viral RNA and free 2',3'-hydroxyl groups in the 5'-terminal m^7G (ref. 10). Consequently, the ^3H -methyl radioactivity bound quantitatively to DBAE-cellulose in buffer A (Fig. 1). Most of the ^{32}P -labelled digestion products consisted of 3' mononucleotides and did not bind; however, 0.08% of the ^{32}P bound to the resin and, after changing the buffer, eluted with the ^3H -labelled $\text{m}^7\text{GpppG}^{\text{m}}\text{pCp}$. The 3'-terminal adenosine released by RNase digestion¹¹ presumably bound but contained no ^{32}P and was undetected. This value is in excellent agreement with the expected amount of DBAE-bound ^{32}P calculated for approximately 35S RNA (7,500 nucleotides) containing a RNase T_2 -resistant, 5'-5'-linked cap structure similar to that in reovirus RNA¹⁰ (5 phosphates).

The RNase T_2 digested mixture of ^{32}P -labelled ASV fragments and ^3H -labelled $\text{m}^7\text{GpppG}^{\text{m}}\text{pCp}$ was eluted from DBAE-

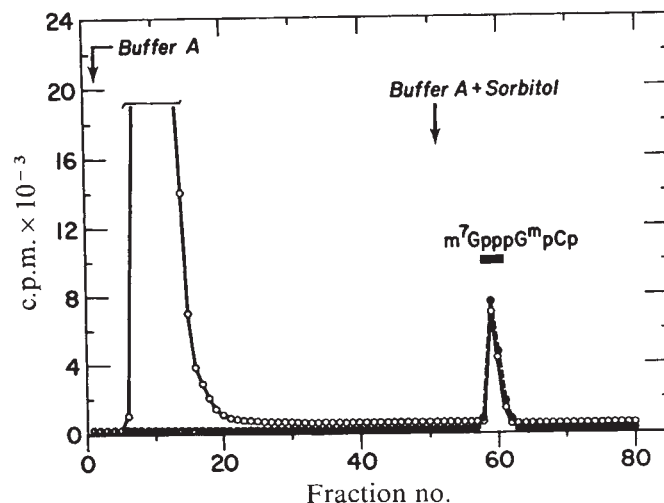


Fig. 1 Chromatography of RNase T_2 digest of ^{32}P -labelled ASV genome RNA on DBAE-cellulose. The B77 strain (sub-group C) of ASV was propagated in chick embryo fibroblasts, isolated by centrifugation and the viral RNA extracted with Pronase-sodium dodecyl sulphate-phenol, all as described previously⁷. Growth medium (phosphate-free) containing dialysed calf serum (see ref. 7 for details) and ^{32}P -orthophosphate (2 mCi ml^{-1}) was changed every 12 h for a total of three collections; viral RNA labelled in this manner had a specific activity of 15×10^6 – 30×10^6 c.p.m. μg^{-1} . The 70S genome RNA was isolated by rate-zonal centrifugation⁷, dissolved in 0.01 M EDTA-0.02 M Tris-HCl, pH 7.4-0.5% (w/v) SDS, heated at 80°C for 4 min and centrifuged through a gradient of 15–30% sucrose containing 0.1 M NaCl-0.01 M EDTA-0.02 M Tris-HCl, pH 7.4-0.5% SDS. Centrifugation was in a Spinco SW 27.1 rotor at 25,000 r.p.m. for 15 h at 20°C . The gradient was fractionated and radioactivity in the fractions measured as Cerenkov radiation. ASV genome RNA, 1.1×10^7 c.p.m., was mixed with ^3H -methyl-labelled reovirus mRNA synthesised *in vitro* ($10\text{ }\mu\text{g}$, 1×10^5 c.p.m.) and digested with 50 units RNase T_2 (Sankyo) in $250\text{ }\mu\text{l}$ of 10 mM sodium acetate buffer (pH 4.5) containing 1 mM EDTA. After incubation for 6 h at 37°C , the reaction mixture was adjusted to starting buffer A (0.05 M morpholine, pH 8.7, 1.0 M NaCl, 0.1 M MgCl_2 , 20% dimethylsulphoxide) in a total volume of 1 ml. The mixture was applied on to a DBAE-cellulose column ($0.6 \times 15\text{ cm}$) equilibrated with buffer A (ref. 9). Elution was carried out with buffer A at a flow rate of 5 ml h^{-1} . When most of the radioactive material had been eluted from the column, the buffer was abruptly changed to buffer A plus 1 M sorbitol and elution continued at a flow rate of $6\text{--}7\text{ ml h}^{-1}$. ^{32}P radioactivity was determined by Cerenkov counting and ^3H radioactivity measured by dissolving an aliquot ($25\text{ }\mu\text{l}$) of each fraction in 1 ml methyl-cellulosolve and 10 ml Aquasol (New England Nuclear). $\circ$, ^{32}P ; $\bullet$, ^3H .

Blocked, methylated 5'-terminal sequence in avian sarcoma virus RNA

mRNAs of many eukaryotic cells and viruses contain blocked, methylated 5'-terminal structures of the type, m^7GpppN (ref. 1). This 'cap' structure, and in particular the 5'-terminal 7-methyl-guanosine, is required for efficient translation *in vitro* of globin, reovirus and vesicular stomatitis virus mRNAs^{2,3}. The genome subunits of RNA tumour viruses probably function as viral mRNA since RNA of the same base sequence is present in virus-specific polysomes of infected cells^{4,5} and virion genome RNA can be translated *in vitro* in a eukaryotic system⁶. Consistent with this possibility, we have found a blocked, methylated 5'-terminal structure— $\text{m}^7\text{GpppG}^{\text{m}}\text{pCp}$ —in the genome RNA of avian sarcoma virus (ASV).

Subunits of ^{32}P -labelled B77-ASV genome RNA were isolated by rate-zonal centrifugation of denatured viral RNA as described previously⁷. RNA from about the 35S region of the gradient migrated in the vicinity of 35S poliovirus RNA when analysed by polyacrylamide gel electrophoresis. The latter procedure resolved the a (35S) and b (smaller than 35S) RNA subunits which correspond to transforming and transformation defective particles⁸ present in the virus stock at levels of 20% and 80% respectively. The RNA hybridised completely with virus-specific DNA synthesised *in vitro* (data not shown).

To test for the presence of blocked 5' termini in ASV genome RNA, we used a procedure developed originally for the isolation of 3'-terminal oligonucleotides from high molecular weight RNA⁹. It depends on the selective affinity of 2'-3' *cis* diols for

Table 1 Methylated nucleosides derived from 70S and 4S ASV RNA

	70S-I	Free 4S	70S-II	35S
m ⁷ G	13*	18	20	2
mC	14	17	21	3
mA	—	1	—	—
mG + mU	36	64	59	10
cap m ⁷ G	5	0	—	14
cap G ^m	5	0	—	
m ⁶ A	27	—	—	
Total c.p.m.	4,227	10,689	2,654	1,048

*Value given in % of total ³H-methyl radioactivity.

Two 100-mm plates of transformed, B77-infected chick cells were incubated for 10 h in 5 ml of amino acid-free medium containing dialysed serum, 10⁻⁴ M each of adenosine and guanosine, and 500 μCi ml⁻¹ of ³H-methyl-methionine (specific activity 10.5 Ci mmol⁻¹, New England Nuclear). The medium was collected, fresh medium of the same composition but lacking radioisotope was added, and collected after 10 h and pooled with the first collection. The virus was collected by pelleting, disrupted in 0.02 M Tris buffer, pH 7.4, containing 0.15 M NaCl, 0.001 M EDTA and 0.5% sodium dodecyl sulphate and the 70S and 4S RNA separated by density gradient centrifugation⁷. The 35S RNA was obtained from 70S RNA after denaturation and centrifugation as described for Fig. 1. The RNA-containing fractions were pooled, precipitated with ethanol, digested with *Penicillium* nuclease and alkaline phosphatase, and analysed by paper electrophoresis at pH 3.5 as described in Fig. 2 (ref. 10). The radioactivity in the region corresponding to methylated derivatives of adenosine in the 70S RNA digest was eluted and identified by paper chromatography with authentic marker N⁶-methyladenosine in two different solvents: isobutyric acid–0.5 M NH₄OH (10:6 v/v) and isopropanol–concentrated NH₄OH–H₂O (7:1:2 v/v). The nuclease- and phosphatase-resistant radioactivity in presumptive caps of 70S RNA was eluted, treated with nucleotide pyrophosphatase and alkaline phosphatase and the digest was analysed by paper electrophoresis and identified as consisting of equal amounts of m⁷G and G^m by subsequent paper chromatography with authentic marker compounds¹⁸. For comparison of the composition of free and 70S genome-associated 4S RNA, values in column (70S-II) were calculated from (70S-I) after omitting the radioactivity in caps and N⁶-methyladenosine. mC, mA, mG, mU are methylated derivatives of the corresponding nucleosides that were not identified further.

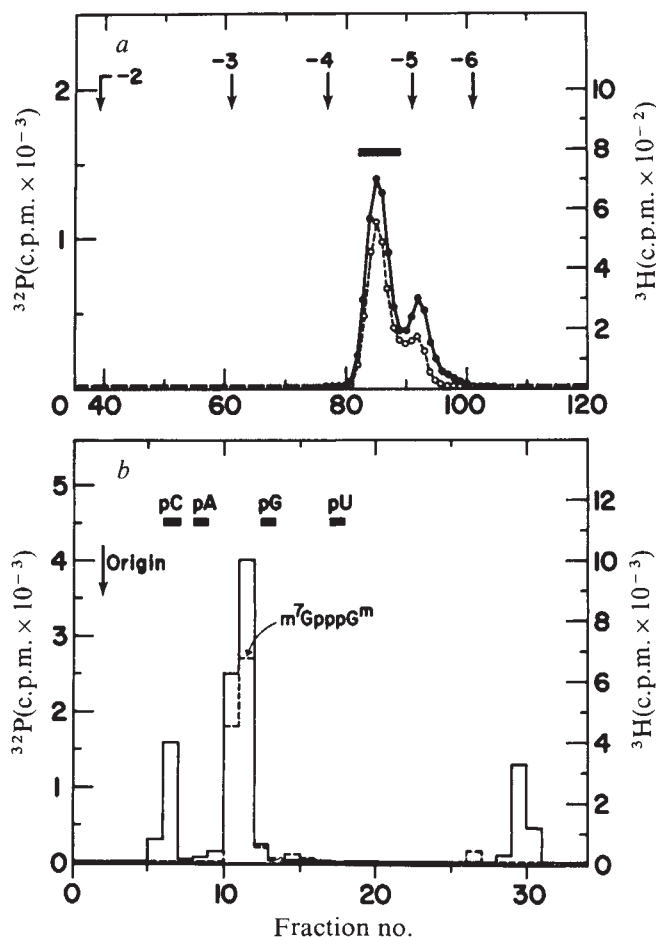


Fig. 2 Characterisation of blocked 5'-terminal fragment from ASV genome RNA. *a*, The peak fractions eluted with buffer A containing 1 M sorbitol (Fig. 1) were diluted tenfold with water and applied to a DEAE-cellulose column (0.6 × 40 cm) equilibrated with 0.02 M Tris-HCl (pH 8.0) and eluted with a linear gradient of NaCl (0.05–0.3 M) in 0.02 M Tris-HCl buffer (pH 8.0) containing 7 M urea (total 150 ml). A pancreatic RNase digest of yeast tRNA was included as marker for the oligonucleotide elution positions. ³²P (●), and ³H (○) radioactivity were determined as described in Fig. 1. *b*, Peak fractions 82–88 were pooled as indicated, desalted, dissolved in 200 μl of 5 mM sodium acetate buffer (pH 6.0) and digested with 100 μg of P₁ nuclease at 37 °C for 30 min. The digest was analysed by high voltage paper electrophoresis in pyridine acetate buffer (pH 3.5) at 50 V cm⁻¹ for 50 min (ref. 10). ³²P, (—); ³H, (---).

cellulose, desalted and reanalysed by DEAE-cellulose chromatography to determine its net charge¹⁰. Two peaks of ³²P with net charges of –4.5 and –5 were resolved, and both contained a corresponding proportion of ³H-methyl radioactivity (Fig. 2). The fractions comprising the major peak were pooled, desalted, digested with *Penicillium* nuclease (P₁) and analysed by paper electrophoresis. P₁ hydrolyses phosphodiester linkages, including those containing 2'-O-methylated residues, to yield 5' mononucleotides from polynucleotides. The enzyme also has 3' phosphatase activity, but cannot cleave cap structures^{10,12}; thus, ³H-methyl-labelled m⁷GpppG^mpCp, derived from reovirus mRNA by RNase T₂ digestion, yielded all the radioactivity as m⁷GpppG^m after P₁ treatment (Fig. 2). By contrast, the ³²P-labelled material yielded radioactivity in the positions of pC, m⁷GpppG^m and Pi in a ratio close to 1:3:1 as expected for P₁ cleavage of m⁷GpppG^mpCp.

Another preparation of RNase T₂ 5'-terminal fragments was digested with P₁ and alkaline phosphatase and analysed by electrophoresis (Fig. 3a). The ³H-methyl-labelled 5'-terminal m⁷GpppG^mpCp was converted to m⁷GpppG^m. The ³²P-labelled fragments yielded 60% of the radioactivity in the position of m⁷GpppG^m and 40% as Pi. The ³H-labelled m⁷GpppG^m and the comigrating ³²P-labelled material were eluted and analysed by descending paper chromatography (Fig. 3b). All the ³H and ³²P radioactivity again comigrated, and the ratio of ³²P to ³H was constant across the peak. The position of the major peak of ³²P corresponded to that of m⁷GpppG^m. The minor peak (fractions 12–14) seemed to result from partial degradation during DBAE-cellulose chromatography at pH 8.7. This material and the smaller peak in Fig. 2a (fractions 90–95) consisted of the ring-opened derivative of m⁷GpppG^m, as determined by electrophoresis and chromatography of nucleotide pyrophosphatase digests. The major peak of radioactivity

(fractions 16–19) was eluted and an aliquot analysed by DEAE-cellulose chromatography; the ³H and ³²P eluted in one peak with a net charge between –2 and –3 (Fig. 3b, inset). The results are consistent with the presence of three phosphates in a phosphatase-resistant structure, previously demonstrated to be m⁷GpppG^m for reovirus mRNA 5' ends¹⁰. Similarly, when ³²P-labelled ASV genome RNA was digested with P₁ and alkaline phosphatase and analysed directly by electrophoresis, 0.04% of the total ³²P was obtained as a peak in the position of m⁷GpppG^m. This value is close to that calculated for one cap structure containing three protected phosphates per RNA molecule of chain length 7,500 nucleotides.

To determine if an identical 5' structure is present in ASV and reovirus RNA, an aliquot of P₁ and alkaline phosphatase-treated mRNA fragments (corresponding to fractions 16–19 in Fig. 3b) was digested with nucleotide pyrophosphatase and analysed by electrophoresis. The ³H-methyl radioactivity in m⁷GpppG^m was converted to equal amounts of m⁷pG and pG^m. The ³²P was resolved into three equally labelled constituents which migrated in the positions of m⁷pG, pG^m and Pi;

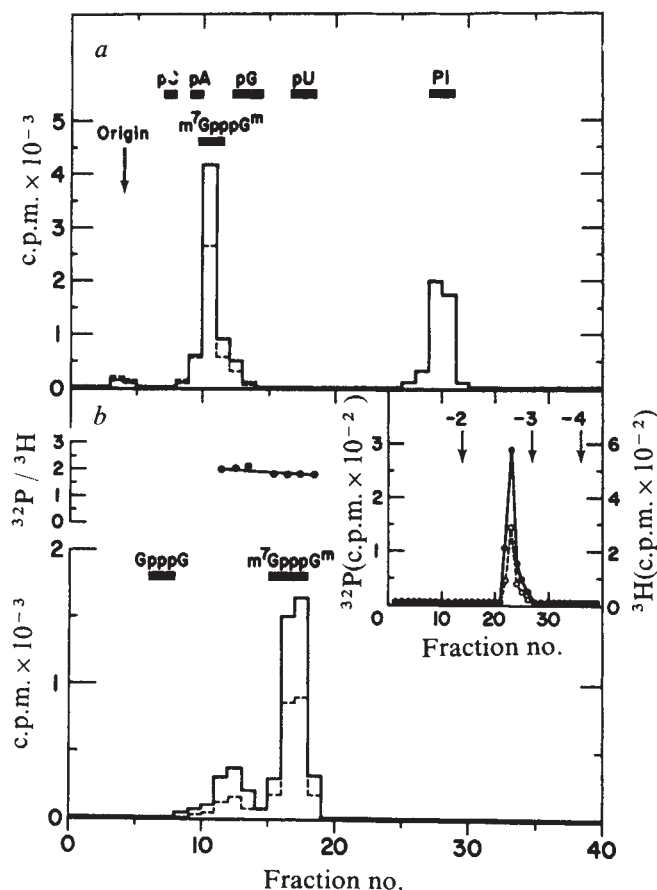


Fig. 3 Analysis of blocked 5'-terminal structure by electrophoresis and chromatography. Another preparation of 5'-terminal RNase T₂ fragments of ³H-methyl-labelled reovirus mRNA and ³²P-labelled ASV genome RNA was obtained as in Fig. 1. a, After desalting, the radioactive DBAE-cellulose-bound material was digested successively with P₁ nuclease and alkaline phosphatase, and the digest analysed by paper electrophoresis; b, the enzyme-resistant radioactivity which migrated between pA and pG was extracted and chromatographed on paper using isobutyric acid-0.5 M NH₄OH (10:6 v/v) as a solvent. Inset, An aliquot of the material in fractions 16-19 of B was applied to a DEAE-cellulose column (0.6 × 25 cm) and eluted as described in Fig. 2a. ○, —, ³²P; ●, ---, ³H.

the nucleotides were eluted and further identified as m⁷pG and pG^m by paper chromatography. Thus the 5' termini of the ASV genome subunits probably have only one type of blocked, methylated structure, namely, m⁷GpppG^mpCp.

In addition to blocked, methylated 5'-terminal structures, poly(A)-containing RNA isolated from eukaryotic cells contains m⁶A in internal positions¹³⁻¹⁸. The same methylated nucleoside was found in ASV genome RNA (Table 1). Virion 70S RNA labelled with ³H-methyl-methionine contained 10% of the total radioactivity in m⁷GpppG^m and 27% in m⁶A, a methylated derivative of adenosine rarely found in tRNA¹⁹. Transfer (4S) RNA present in virions but not associated with the genome RNA contained no cap structures and only a low level of methylated adenosine; m⁷G and methylated derivatives of cytidine, guanosine and uridine were present as reported previously for 4S RNA in Rous sarcoma virus²⁰. The proportions of methylated nucleosides in free 4S RNA and 70S RNA were similar when calculated after subtracting the radioactivity in caps and m⁶A, consistent with the presence of the latter in genome subunit RNA (Table 1). Direct analysis of ³H-methyl-labelled approximately 35S RNA from denatured 70S RNA revealed that ASV genome RNA contained 71% of the total radioactivity in m⁶A and 14% in m⁷GpppG^m, sug-

gesting that there are about 10 residues of m⁶A per RNA subunit. On the basis that 4S RNA contains an average of 11 methylated nucleotides¹⁹, the number of 4S RNA molecules per genome subunit was estimated to be close to two.

ASV genome RNA, like cellular cytoplasmic mRNA, contains 5'-m⁷GpppN, 3'-terminal poly(A) and internal m⁶A and is probably formed by cellular mechanisms of mRNA biosynthesis. Poly(A)-containing RNA from the nuclei of HeLa cells also has m⁷GpppN at the 5' end¹⁸. It is not, however, known whether ASV genome RNA is 'capped' and methylated in the nucleus or in the cytoplasm of infected cells. Viral RNA may be made from larger precursor RNA by a processing mechanism involving concomitant addition of caps²¹. Alternatively, 'capping' of ASV genome RNA may occur at the initiation step of transcription in a manner analogous to mRNA synthesis and modification by virion-associated enzymes²²⁻²⁴. The same 5'-terminal sequence, m⁷GpppG^mpCp, has also been found in Prague strain Rous sarcoma virus 35S RNA (J. M. Keith and H. Fraenkel-Conrat, personal communication).

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Thermodynamic activation parameters of fish myofibrillar ATPase enzyme and evolutionary adaptations to temperature

INTERSPECIFIC compensatory adaptations to environmental temperature which occur at the molecular level have been demonstrated for several enzyme systems¹. Most of these studies have been concerned with either kinetic parameters such as K_m (refs 2, 3) or thermodynamic parameters such as activation energy^{2,4}. The significance of changes in these parameters in the overall mechanism of evolutionary temperature

Table 1 Thermodynamic activation parameters for fish myofibrillar ATPase activity

Species	Approximate environmental temperature (°C)	Assay temperature (°C)	V _{max} (μmol Pi mg ⁻¹ min ⁻¹)	E _a (calorie mol ⁻¹)	No. assays	ΔH [‡] (calorie mol ⁻¹)	ΔS [‡] (entropy units)	ΔG [‡] (calorie mol ⁻¹)
<i>Nothenia rossii</i>	South Georgia, British Antarctica (0°–2 °C)	0.5	0.24			6,850	–42.2	18,450
		18	0.81	7,400**	21	6,800	–39.4	18,300
<i>Gadus virens</i>	North Sea (5°–14 °C)	0.5	0.06			10,200	–33.7	19,400
		18	0.82	10,700*	18	10,100	–28.1	18,900
<i>Gadus morhua</i>	North Sea (5°–14 °C)	0.5	0.07			11,350	–26.2	18,500
		18	0.61	11,900***	24	11,300	–24.6	19,050
<i>Amphiprion sebae</i>	Indian Ocean (about 23°–25 °C)	0.5	0.013			16,750	–9.6	19,400
		18	0.57	17,300*	18	16,700	–6.1	18,500
<i>Carassius carassius</i>	Domestic (Acclimatised to 26 °C)	0.5	0.03			21,400	8.3	19,050
		18	0.35	21,900*	18	21,300	8.6	18,800
<i>Tipalia nigra</i>	Equatorial African freshwater lake (23°–31 °C)	0.5	0.017			31,000	42.9	19,300
		18	0.045	31,600**	18	31,500	42.5	18,600
<i>Tipalia grahami</i>	Equatorial hot springs soda lake (35°–38 °C)	0.5	0.016			32,800	49.2	19,300
		18	0.69	33,300***	18	33,300	49.2	19,000

Myofibrils were prepared from the dorsal epaxial musculature as before¹¹, care was taken to exclude superficial red muscle as this has a different myofibrillar ATPase activity¹². The assay for ATPase activity was performed in 1.5 ml of 40 mM Tris-HCl (pH 7.5) with 6 mM ATP, 6 mM MgSO₄ and 0.2 mM CaCl₂ at $I = 0.12$ (adjusted with KCl) and at a myofibrillar concentration of 0.4–0.5 mg ml⁻¹. The reaction was terminated by addition of TCA and the Pi liberated was determined^{13,14}. Appropriate controls and reagent blanks were included in all experiments. Determinations of myofibrillar ATPase activity were made in triplicate at a series of temperatures between 0° and 18 °C. Activation energies (E_a) for the reactions over this temperature range were calculated from the corresponding Arrhenius plots. The Arrhenius plots were found to be linear within this temperature range for all species studied (statistical analyses given above). Thermodynamic parameters were calculated according to the following relationships¹⁵: $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$; $\Delta H^{\ddagger} = E_a - RT$; $\Delta S^{\ddagger} = 4.576(\log K - 10.753 - \log T + E_a/4.576T)$ and $K(s^{-1}) = V_{max}$ per mg of enzyme $\times$ molecular weight $\times 10^{-3}$ mmol μmol⁻¹ $\times 1$ min per 60 s, where the molecular weight is expressed as mg mmol⁻¹ and V_{max} as μmol mg⁻¹ min⁻¹. The proportion of myosin in the myofibril was assumed to be 54% (ref. 7), with a molecular weight of 240,000 per enzyme site^{16,17}. All protein determinations were carried out using a Biuret method¹⁸.

* $P = 0.01$.

** $P = 0.005$.

*** $P = 0.001$.

compensation is controversial¹. In the case of activation energy (E_a), as calculated from Arrhenius' equation, a correlation exists with habitat temperature for some enzymes^{2,5,6} but not others³. Studies of activation energy are principally concerned with the enthalpy of activation (ΔH[‡]). There have been comparatively few studies of the free energy of activation (ΔG[‡]) between homologous enzymes from animals of different thermal environments^{7,8}. Low *et al.*⁸ showed a correlation between ΔG[‡] for muscle type (M₄) lactate dehydrogenase and body temperature. The relative importance of enthalpic (ΔH[‡]) and entropic (ΔS[‡]) activation between poikilotherms and homeotherms was also shown to be different⁸. Similar results have been obtained for skeletal muscle myofibrillar ATPase activity⁷. Since these studies deal with homologous enzymes from animals with very different phylogenetic positions it is difficult to assess directly the adaptive significance of changes in the magnitude of these parameters.

We have determined thermodynamic activation parameters for the Mg²⁺-activated myofibrillar ATPase of the white muscle of teleost fish inhabiting a wide range of thermal environments from Antarctic to tropical waters.

A plot of log₁₀V_{max} against the reciprocal of the absolute temperature (K) is called an Arrhenius plot. Arrhenius plots for myofibrillar ATPase activity show a discontinuity at 18.5 °C (ref. 7). This effect has been attributed to the binding of Ca²⁺ to troponin A in systems containing the intact calcium-sensitising system, since it does not seem to be observed with pure myosin in the presence of high levels of Ca²⁺ or with desensitised actomyosin preparations⁹. In the case of some species of fish, myofibrillar ATPase activity also undergoes an initial activation before denaturation at higher temperatures^{6,10}. This initial activation effect makes accurate determinations of native specific activity very difficult¹¹. Although the occurrence of this initial activation varies considerably between species (occurring at lower temperatures in cold-adapted species⁹) it is not observed in any of the species studied at temperatures below 25 °C. To overcome these complications, we have considered only the temperature range 0–18 °C. The method of calculating the thermodynamic parameters,

ΔH[‡], ΔS[‡] and ΔG[‡] from the corresponding Arrhenius plots and V_{max} determinations is given in the legend to Table 1.

Values of ΔG[‡], the free energy of activation of the reaction, were broadly similar for all species studied. The values obtained for the Antarctic fish *Nothenia rossii*, however, were some 800 calorie mol⁻¹ lower than those of species living at the highest environmental temperature (Table 1). Small differences in ΔG[‡] between homeotherms and poikilotherms have been noted both for myofibrillar ATPase⁷ and M₄ type muscle lactate dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase and muscle glycogen phosphorylase b⁸, and may be of some adaptive significance. Table 1 shows that a more important feature of temperature adaptation in this enzyme concerns the relative contributions of enthalpic and entropic activation. While ΔG[‡] was relatively constant between species the proportions of ΔH[‡] and ΔS[‡] varied considerably. A positive relationship between entropy of activation and environmental temperature was demonstrated. Values of ΔS[‡] varied from large negative values for the cold-adapted species to high positive values for the tropical species. Similar differences have been found between the entropy terms of the myofibrillar ATPase from birds and mammals where entropy was high and positive relative to amphibians and reptiles, where the values were low or negative⁷. There was also a strong positive correlation between enthalpy of activation and the mean annual habitat temperature of the species. Values for ΔH[‡] varied from 6,850 calorie mol⁻¹ for the Antarctic species (*Nothenia rossii*) (mean water temperature 0–2 °C) to 33,000 calorie mol⁻¹ for a species from an equatorial hot springs soda lake, *Tipalia grahami* (35–38 °C). Both enthalpies and entropies of activation were fairly independent of assay temperature.

A compensation plot¹⁹ of entropy change (ΔS[‡]) against enthalpy change (ΔH[‡]) for the different species was highly linear ($P < 0.001$) (Fig. 1). The slope of this plot has the dimensions of K and is called a proportionality constant or compensation temperature¹⁹. Our values of 280 and 300 K for assay temperatures 0.5 and 18 °C respectively are in the range reported for similar plots for a wide range of protein

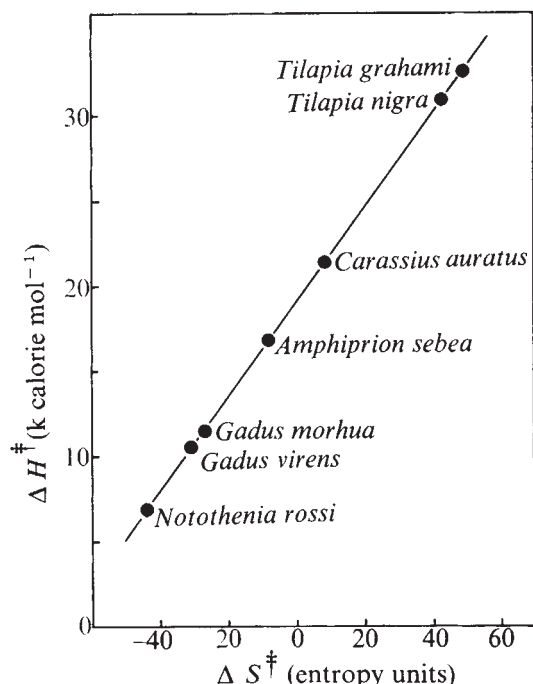


Fig. 1 The relationship between the enthalpy of activation and the entropy of activation for a range of species of fish living at different environmental temperatures.

reactions¹⁹⁻²². Examples include the binding of oxygen to vertebrate haemoglobin²⁰ and the formation of methaemoglobins for several mammalian species^{21,22}. The existence of a similar relationship between entropy change and enthalpy change, yielding similar compensation temperatures, for various processes of small solutes in water solution, has led some authors to implicate the participation of liquid water in these protein reactions^{19,20}. At present it is not clear whether the linear compensation patterns of these and other protein reactions are attributable to some general feature of protein-water interactions or arise entirely from some properties of the protein molecules themselves. If such compensation behaviour was found to indicate a general water-based phenomenon in protein reactions, this would have considerable biological importance in providing a theoretical framework both for the study of specific physiological processes and in the elucidation of mechanisms of molecular evolution.

Certainly, compensation behaviour of this type seems to provide biologically useful adjustments in the activation entropies and enthalpies for poikilothermic enzymes. The observed changes in $\Delta H^\ddagger$ and $\Delta S^\ddagger$ for myofibrillar ATPase, both of which increase from cold water to tropical habitats, are reflected in corresponding changes in the heat and entropy content of the muscle cells. Fish living at the higher environmental temperatures, where enthalpic activation is presumably facilitated, show a larger contribution of the enthalpic activation component. At low habitat temperatures, however, where enthalpic activation is likely to be energetically more unfavourable, the enthalpic component is partially replaced by a larger entropic contribution to the free energy of activation (Table 1). This has the advantage of greatly reducing the temperature sensitivity of the rate-limiting step in forming the activated enzyme-substrate complex. It might be expected that this would give significant adaptive advantage both to species which experience large fluctuations in environmental temperature and to those living in cold and temperate waters, since the limitations of the low heat content of the cellular environment have been partly offset.

It is known that myosins isolated from cold water species are characterised by a ready formation of aggregated products, within a few hours of preparation, and a concomitant decline of ATPase activity to zero²³. Such preparations are also more

easily denatured by urea and heat than the corresponding myosins from homeotherms²⁴. It seems therefore that the concomitant structural adaptations in the tertiary structure of fish myosins consistent with their evolutionary modification for a low enthalpy environment necessarily make the molecule unstable at higher temperatures. Indeed a striking relationship has been shown between the thermostability of fish myofibrillar ATPase activity and the environmental temperature at which the species lives²⁵. The general order of thermal denaturation for this enzyme at 37 °C from 22 species of fish has been shown to be African equatorial lakes > Indian Ocean > Mediterranean > North Sea > Antarctic^{6,25}. The approximate half life of inactivation of the enzyme in comparable conditions varied some 350 times between these two temperature extremes. Species adapted to tropical environments presumably need a more rigid molecular structure to confer thermal stability at the higher environmental temperatures. Somewhat less marked correlations between thermostability and thermal environment have been shown for several mitochondrial enzymes in teleosts²⁶.

In view of the relevance of studies of myofibrillar ATPase to an understanding of muscle contraction, the molecular mechanisms of temperature compensation among this group of proteins seem to be of particular interest. A more detailed account of these phenomena will be presented later.

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C₄ photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*

GREEN plants may be divided into two groups, C₃ and C₄ species, depending on whether the first product of photosynthetic CO₂ fixation is phosphoglycerate (C₃) or oxaloacetate (C₄). In all species studied to date the C₄ metabolism is associated

with either 'Kranz' leaf anatomy, low CO_2 compensation points or low $^{13}\text{C}/^{12}\text{C}$ ratios. Included in the C_4 category are many of the highest yielding and rapidly growing crops, most of which are believed to have originated in tropical and subtropical regions. C_4 species so far studied, which are mostly of tropical origin, attain their highest photosynthetic rates at leaf temperatures of about 30°C and above^{1,2} so that selective advantages derived from photosynthetic rate will only be evident in tropical and subtropical regions^{1,2}. A recent compilation of the reported rates of photosynthesis and productivity of C_3 and C_4 crops³ showed that differences in maximum annual dry matter yields could be explained by differences in lengths of growing season. It thus remains to be shown that there is a selective advantage which is a direct consequence of photosynthetic capacity of C_4 species or that there is an inherent physiological barrier to the adaptation of C_4 species to a similar range of latitudes to C_3 species.

There are two indirect consequences of C_4 photosynthesis which could be advantageous to plants irrespective of the climate in their region of origin. First, the ratio of weights of CO_2 assimilated to water transpired (the water-use efficiency) of C_4 plants, which is often twice that of C_3 species⁴, will be advantageous in habitats where water supply is limiting for growth or survival. Second, tolerance of salinity is a conspicuous attribute of many C_4 species. Indeed sodium is an essential micronutrient specific to C_4 species⁵ and may increase the *in vivo* activity of the primary carboxylating enzyme of C_4 photosynthesis, phos-

Fig. 1 Transverse section of a leaf of *Spartina townsendii* $\times 740$. The material was fixed in glutaraldehyde and post fixed in osmium tetroxide; after dehydration in ethanol, it was embedded in Epon and 1- μm sections cut for light microscopy²⁸. This section was stained with Toluidine blue and mounted in immersion oil. X, Bundle sheath cell containing chloroplasts. ('Kranz', the German word for wreath, was first used by Haberlandt⁷ to describe two concentric layers of chlorenchymatous tissues round the vascular bundles, the inner being the bundle sheath which contains chloroplasts, distinct from those of the outer mesophyll.)

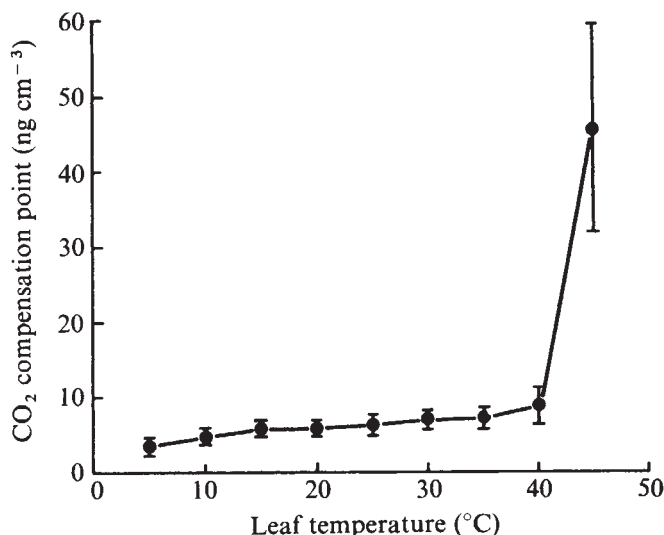
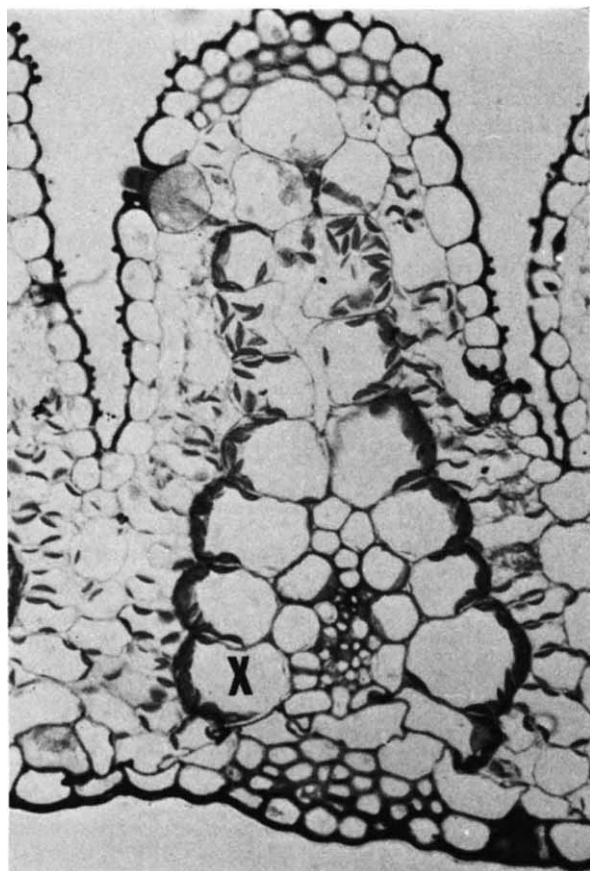


Fig. 2 Mean $\pm$ s.e. of CO_2 compensation point for leaves of *Spartina townsendii* at nine leaf temperatures and photon flux density of about $200 \text{ nmol cm}^{-2} \text{ s}^{-1}$. The compensation points were determined using an infrared gas analyser (Analytical Development, Series 225) in a simple closed-circuit system. The analyser was calibrated in the range $0\text{--}100 \text{ ng cm}^{-3}$ using mixtures generated from pure N_2 and 1% CO_2 (Rank Precision Industries) with two gas mixing pumps (H. Wösthoff) in series. The concentration of the 1% standard was checked against mixtures generated from pure CO_2 and pure N_2 , and cross-checked by conductivity analysis²⁹. In practice it was found possible to discriminate differences of less than 0.2 ng cm^{-3} over this calibration range. Photon flux density at the leaf surface was determined using a Quantum sensor (Lambda Instruments)³⁰. Leaf temperature was determined with a copper-constantan thermocouple junction (diameter 0.1 mm) tightly appressed to the lower surface of the leaf.

phenol-pyruvate carboxylase⁶. We therefore searched for temperate C_4 species in physiologically dry and saline habitats.

Three out of thirty common higher plant species, collected from coastal sand dunes and salt marshes around the coast of Britain, were found to have the 'Kranz' leaf anatomy associated with C_4 photosynthesis: *Salsola kali* L., *Atriplex lacinata* L., and *Spartina townsendii* (*sensu lato*). *S. kali* has previously been noted to be a C_4 species⁸. *A. lacinata* and *S. kali* occur as far north as Sweden and are locally abundant at many sites around the British coast. *S. townsendii* is exceptional among the C_4 species now known to be native or introduced to Britain, in that it is a dominant component of large areas of our coastal salt marshes⁹.

Previous studies of *S. townsendii* have reported the bundle sheath to consist of large colourless cells^{10,11}, or only to contain chlorophyll in young plants¹². All material examined in populations from Southampton Water, the Ribble Estuary, and the Humber Estuary, however, showed 'Kranz' leaf anatomy (Fig. 1). In all cases the large bundle sheath cells contained numerous chloroplasts, often adjacent to the outer wall. Only the chloroplasts of the bundle sheath accumulated starch; they also differed in having twice the cross-sectional area and grana with fewer thylakoid stacks. All of these differences between chloroplasts have been found to a greater or lesser extent in the leaves of other C_4 species¹³, and clearly establish the occurrence of 'Kranz' anatomy in *S. townsendii*.

To test the anatomical inference that *S. townsendii* is a C_4 species we determined the CO_2 compensation point—that is the CO_2 concentration in air at which the net flux of CO_2 at the leaf surface in the light is zero. This is usually within the range $0\text{--}20 \text{ ng cm}^{-3}$ for C_4 species and $60\text{--}140 \text{ ng cm}^{-3}$ for C_3 species. The mean CO_2 compensation point for *S. townsendii* does not exceed 10 ng cm^{-3} over the range of leaf temperature $5\text{--}40^\circ\text{C}$ (Fig. 2) which confirms the occurrence of C_4 photosynthesis.

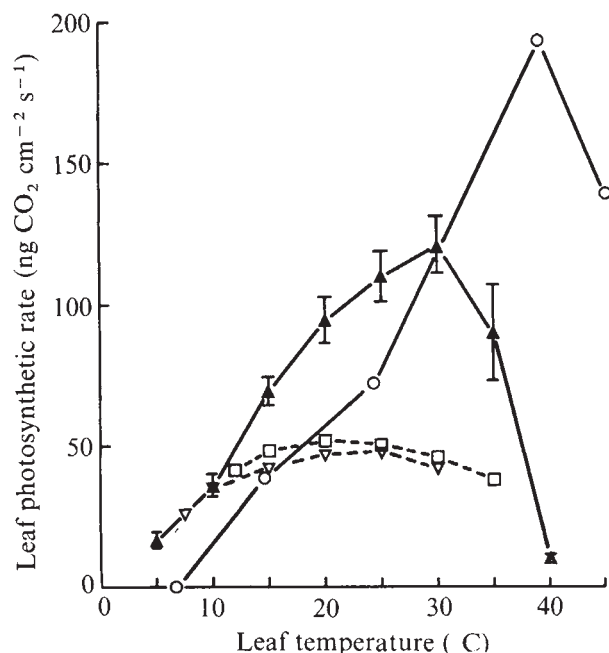


Fig. 3 Mean $\pm$ s.e. of response of leaf photosynthetic rate at a photon flux density of about $200 \text{ nmol cm}^{-2} \text{ s}^{-1}$, to leaf temperature for *S. townsendii* (▲) compared with data obtained by others for three other grass species. Plants of *S. townsendii* collected from the Humber Estuary were grown in a controlled-environment cabinet (CM94PG, Fisons) at day-night air temperatures of 16/13 °C, a vapour pressure deficit of about 0.5 kPa and a photon flux density during the 16-h day of $50 \text{ nmol cm}^{-2} \text{ s}^{-1}$ at the crop surface. Nutrient solution (200 ml) (Solu-feed, ICI) was added to each 15-cm diameter plant pot once every 3 weeks. Steady-state leaf photosynthetic rate was determined in an open-circuit gas exchange system. A glass water-jacketed, air-sealed, leaf chamber was used to provide accurate environmental control over single attached leaves. The light source was a high pressure sodium lamp (SON/T 400W, Philips) with a parabolic reflector. Photon flux density and leaf temperature were measured as described in Fig. 2. Ambient CO_2 concentration in the air supplied to the leaf was maintained at 600 ng cm^{-3} , and the water vapour pressure deficit at 0.3 kPa. The net flux of CO_2 at the leaf was determined by differential gas analysis. The infrared gas analyser was calibrated by the split-tube method of Parkinson and Legg³¹ using gas mixtures generated by the method described in Fig. 2. Growth of material and measurement of photosynthesis and microclimate for *Sesleria albicans* (▽; ref. 15), *Festuca arundinacea* cv. S170 (□; ref. 16) and *Pennisetum purpureum* cv. 05088 (○; ref. 14) was by similar methods to those described here.

Although many C_4 species may attain leaf photosynthetic rates often three times those of C_3 species, these differences are only realised at relatively high temperatures. The C_4 species, *Pennisetum purpureum*, is reputed³ to be the world's highest yielding forage crop. At 38 °C its rate of leaf photosynthesis¹⁴ is some three times the maximum obtained by two temperate C_3 grasses *Sesleria albicans*¹⁵ and *Festuca arundinacea*¹⁶ (Fig. 3). At 16 °C, however, the mean air temperature for the warmest month in South-east Britain, its rate is lower than that obtained by the C_3 grasses. In common with other C_4 grasses, photosynthesis in *P. purpureum* ceases at leaf temperatures of 7 °C and below, whereas *S. townsendii* maintains significant photosynthetic rates at 5 °C (Fig. 3) and rates at relatively low temperatures, 5–10 °C, comparable with C_3 grasses (Fig. 3). The leaf photosynthetic rate of *S. townsendii* at 25 °C is about 50% higher than the maximum rates reported for the major herbage grasses of Britain¹⁷. Its temperature optimum for photosynthesis (30 °C) however, is 7–11° lower than optima for other C_4 grasses, measured in similar conditions^{14,18,19}. Net

photosynthetic rate in *S. townsendii* declines with increase in temperature above 30 °C, so that at 40 °C it is 10% of the rate at 30 °C. This does not result from the occurrence of photorespiration at these temperatures, because the compensation points from 30–40 °C do not differ significantly from those at lower leaf temperatures (Fig. 2).

S. townsendii is considered to have arisen from the hybridisation of *S. maritima* (Curt.) Fernald, endemic to Europe and Africa, and *S. alterniflora* Lois., a species introduced from North America²⁰. *S. alterniflora* is one of five species of the genus *Spartina* native to North America which have previously been suggested to be C_4 (refs 21–25). *S. maritima* (Mersea Island, England) possessed typical 'Kranz' leaf anatomy. Since its first recorded appearance at Southampton Water in 1870, *S. townsendii* has spread, both by natural dispersal and by deliberate plantings, to occupy over 12,000 hectares of intertidal mudflats around the British Isles. Attempts to introduce *S. townsendii* to latitudes below 48 °N have repeatedly failed, but it has been successfully introduced to Udale Bay, Scotland (57.5 °N), and to salt marshes in Denmark where it now occupies over 500 hectares (ref. 26). This pattern of distribution suggests that *S. townsendii* is a plant limited to cool temperate regions and thus affords an unrivalled opportunity to examine the potential of C_4 photosynthesis in these conditions.

It is of interest that the perennial *Spartina gracilis* Trin., an inland North American species, which has a distribution extending to above 60 °N into the North West Territories of Canada²⁷, also has 'Kranz' leaf anatomy.

In conclusion, *S. townsendii* is a C_4 species adapted and limited in distribution to cool temperate climatic regions. One feature of this adaptation is the ability of the species to maintain significant rates of photosynthesis at relatively low temperatures, which contrasts with observations on other C_4 grasses. Thus, C_4 photosynthesis is not inherently limited to subtropical and tropical regions and physiological adaptation of this process to a cool temperate habitat has occurred.

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X-ray evidence of temperature-dependent conformational disorder of hydrogen-bonded carboxylic acid molecules in crystal structures

FROM an analysis of X-ray data on carboxylic acids¹ we have established the stability of the approximately coplanar arrangement of the non-hydrogen atoms of the acetic acid group (C-COOH) with the α substituent in α -substituted carboxylic acids (Fig. 1). In the crystal structures of tartaric acid (X = OH) (ref. 2), aminomalonic acid (X = NH₂) (ref. 3) and fluoroacetic acid (X = F) (ref. 4) the coplanar *cis* conformation has been found. In crystals of unsubstituted monocarboxylic acids the acetic acid group has been found to be coplanar with the β -carbon atom¹.

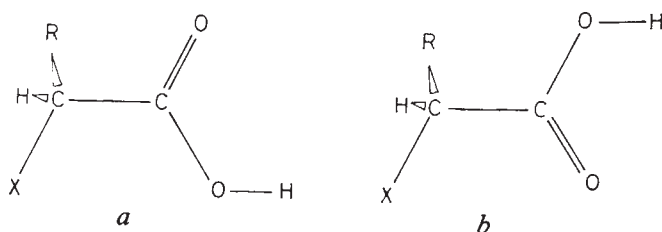


Fig. 1 *Trans* (a) and *cis* conformation (b) in α -substituted carboxylic acids.

From infrared studies of unsubstituted monocarboxylic acids at different temperatures, Hayashi *et al.*^{5,6} deduced that in odd-membered acids the crystal contains the *cis* form at lower temperatures, whereas (lower members excepted) crystals of the even-membered acids contain the *trans* form. As yet, X-ray evidence to support these conclusions is scarce⁷⁻⁹. Hayashi also pointed out that by assuming the difference in energy between the *cis* and *trans* forms to be small, it may be expected that they coexist in crystals of carboxylic acids in various temperature-dependent statistical proportions.

A feature common to the crystal structures of carboxylic acids is that the carboxyl groups predominantly couple with one another to form the well known eight-membered rings. A transition from the *trans* to the *cis* form or *vice versa* may be conceived of as a simultaneous transport of the protons along the hydrogen bond, accompanied by a rearrangement of the electron density and subsequent changes of bond geometry in the various carbon-oxygen bonds, packing considerations ruling out the mechanism involving a rotation of the carboxyl groups (Fig. 2). The assumed coexistence of the *trans* conformation and that of *cis* in different ratios in crystals will imply that in the X-ray analysis C-O bond lengths and C-C-O bond angles are found that are weighted averages of C=O and C-O lengths and C-C-O and C-C=O angles, respectively.

This variation in the carboxyl group geometry is, as far as bond lengths go, subject to the Speakman rule¹⁰, which states that the sum of the carbon-oxygen distances will be 2.52 ± 0.02 Å. For example, at room temperature (RT) C-O bond lengths in benzoic acid¹¹ are 1.24 and 1.29 Å, whereas in tartaric acid² these distances are 1.21 and 1.30 Å.

It is in the RT structure of fluoromalonic acid¹², where C-O lengths and C-C-O angles are equal within experimental error (Table 1), that this supposed *cis-trans* disordering must be at its maximum. The expectation that such a disorder would split

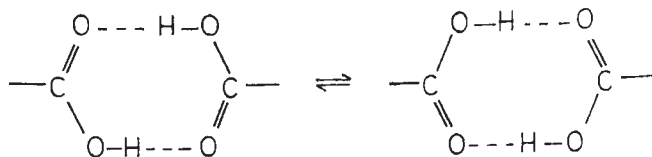


Fig. 2 *Cis-trans* transition by proton transfer in a cyclic carboxylic acid dimer configuration.

the hydrogen atom electron density along the hydrogen bond donor-acceptor line, we found to be true in a subsequent, more accurate redetermination of the crystal structure of fluoromalonic acid (Fig. 3a). Hayashi's concept of temperature-dependent disordering in crystals of organic acids prompted us to look into the change in carboxyl group geometry at liquid nitrogen temperature (LNT) in crystalline fluoromalonic acid. As this acid constitutes the ideal test case, a temperature decrease here dictates a drastic alteration of disordering.

X-ray results derived from data obtained from a single crystal of the highly hygroscopic acid, prepared by sublimation *in vacuo*, are summarised in Table 1. Cooling selectively reduces the length of the *b* axis by about 0.4 Å, whereas the *c* axis increases by about 0.09 Å. The carboxyl group geometry tends to be similar to that of a 'normal' carboxyl group. The hydrogen atom electron density splitting has disappeared and

Fig. 3 Electron density difference synthesis in the plane of the carboxyl group dimer. Contours on an arbitrary scale. a, RT; b, LNT.

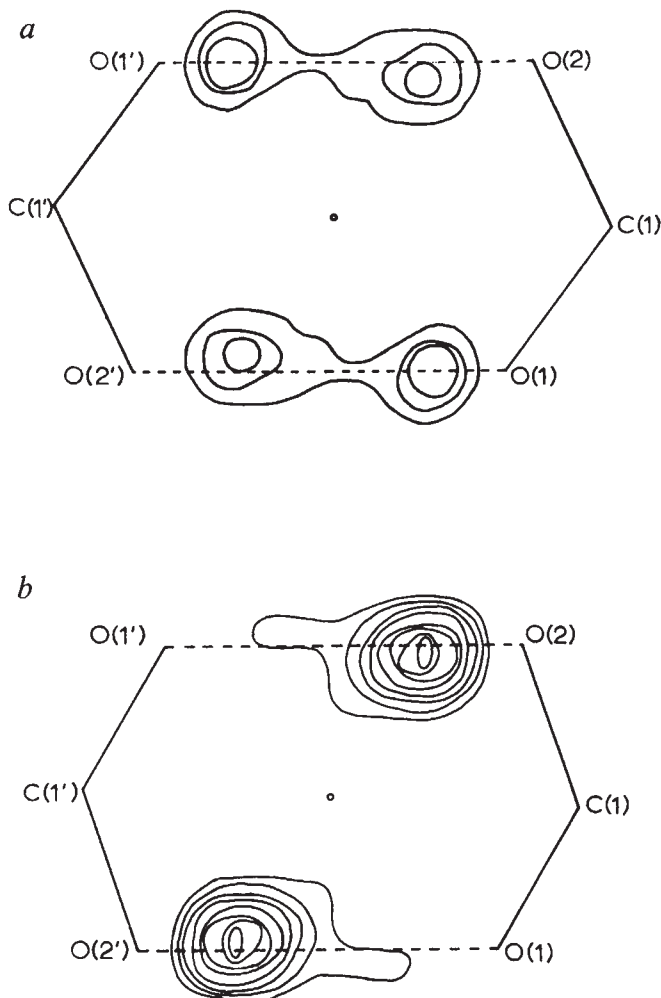


Table 1 Summary of X-ray crystal structure analysis of fluoromalonic acid at RT and LNT

	RT	LNT
Cell dimensions	$a = 4.593 \text{ \AA}$ $b = 8.322$ $c = 11.214$	4.568 $\text{\AA}$ 7.930 11.307
Radiation	Cu(K α)	Cu(K α)
Number of reflections	415	398
Space group	Pnam ($Z = 4$)	Pnam ($Z = 4$)
R factor	0.032	0.042
$R = \frac{\sum F_o - F_c }{\sum F_o }$		
C(1)–O(1)	1.251(2) $\text{\AA}$	1.224(2) $\text{\AA}$
C(1)–O(2)	1.254(2)	1.291(2)
C(2)–C(1)–O(1)	116.5(2)°	119.5(2)°
C(2)–C(1)–O(2)	117.1(2)	114.1(2)
O(1)–C(1)–O(2)	126.4(2)	126.4(2)
C(1)–C(2)	1.523(2) $\text{\AA}$	1.529(2) $\text{\AA}$
C(2)–F	1.367(3)	1.373(4)
C(2)–H(1)	0.96(3)	0.95(4)
O(2)–H(2)	Disordered	0.88(3)
C(1)–C(2)–C(1')	111.4(2)°	111.1(2)°
C(1)–C(2)–F	110.1(1)	110.5(2)
C(1)–C(2)–H(1)	108(1)	107(1)
H(1)–C(2)–F	109(2)	109(2)
C(1)–O(2)–H(2)	Disordered	105(2)
Hydrogen bond: O(1)–O(2')	2.657(2) $\text{\AA}$	2.649(2) $\text{\AA}$

(') = $1 - x, -y, -z$.

an asymmetric single maximum is now found in the electron-density difference map (Fig. 3b). As the molecule at LNT clearly has the *trans* form, the question arises as to whether the established *cis* stability at RT in crystals of α -hydroxyacids and of fluoroacetic acid also applies to these structures at LNT.

The results for fluoromalonic acid give the first unambiguous X-ray evidence of conformational disorder at elevated temperatures in molecular crystals of carboxylic acids. Note that the RT X-ray analysis of the crystal in question after the LNT experiment again shows the same geometrical anomalies, which can now definitely be ascribed to conformational disorder.

We thank Dr Hayashi for making available his results before publication.

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Errata

In the article "The modulation contrast microscope" by R. Hoffmann and L. Gross (*Nature*, **254**, 586; 1975) equation (1) should have read

$$U_{(0)} \sim \int e^{-i\phi^x} e^{-i\theta^x} dx$$

and not as printed.

In the article "Localisation of human peptidase-A structural locus from studies on a cultured lymphoblastoid line" by E. Arthur, C. M. Steel, H. J. Evans, S. Povey, B. Watson and H. Harris (*Nature*, **257**, 308; 1975) the legends for Figs 2 and 3 were transposed. The figures are correct as they stand.

Corrigendum

In the article "A resonant point absorber of ocean-wave power" by K. Budal and J. Falnes (*Nature*, **256**, 478; 1975) the absorption length $d_a = P_{opt}/K$ was estimated to be $d_a = 50$ m in small-amplitude sinusoidal waves and $d_a = 20$ m in real North Atlantic waves when the tank diameter is $2a = 16$ m. This result was based on the approximation that diffraction effects are negligible when $a \ll \lambda$ where λ is the wavelength. Unfortunately, this approximation overestimates d_a when a is as large as 8 m. Including diffraction effects in the calculation it is found that the absorption length in small-amplitude sinusoidal waves is $d_a = \lambda/2\pi$, irrespective of the value of a . This result is valid for any circularly symmetrical, optimised, resonance-tuned heaving body on deep water. In small-amplitude sinusoidal waves of period $T = 10$ s this gives $d_a = 25$ m instead of $d_a = 50$ m. In the case of wind-generated waves the overestimation of d_a is less drastic. The general conclusions drawn regarding the advantage of point absorbers are still valid.

reviews

OVER the last decade the quest for a semantics of natural language has reached the proportions of an intellectual's crusade. Dedicated regiments of linguists, logicians, psychologists, and computer scientists, have set off for the Holy Land, and massive amounts of intelligence, both real and artificial, have been directed at what turns out to be a most intractable infidel. There have been a few piecemeal victories, but the campaign so far has been a failure. There is no single hypothesis that provides a convincing and general theory of what meaning is.

Walter Kintsch's book offers us a psychologist's view of the battleground, and it illustrates many of the strengths and most of the weaknesses of this perspective. Kintsch believes that the essential strategy is to find the right notation. He represents the meaning of a sentence such as, "Peter put the book on the table", by the formula: (PUT, AGENT: PETER, OBJECT: BOOK, GOAL: TABLE).

That is all very well, but what is the notation for; what does it do? Kintsch does not tell us. Nor does he tell us precisely what he means by AGENT, OBJECT, or GOAL. Indeed, one of the great drawbacks of these notions, which derive from Fillmore's 'case' grammar, is that no one has ever succeeded in stipulating a meaning for them that is wholly explicit and that requires no exercise of intuition on the part of the reader. The trouble with doing semantics by translating an expression from one language into another is that one needs a semantics for the 'meta'-language. Kintsch does not provide one, and so it is singularly unsatisfying to learn that the meaning of 'put' is 'PUT'. But at least at this point we reach a current controversy.

GOAL: MEANING

P. N. Johnson-Laird

The Representation of Meaning in Memory. (The Experimental Psychology Series.) By Walter Kintsch. Pp. vii+279. (Lawrence Erlbaum: Hillsdale, New Jersey, 1974.) £8.00.

Everyone agrees that the meanings of many words can be decomposed into more basic constituents: 'kill' can be analysed, for example, as 'cause to become not alive'. The question is: should this analysis be part of the meaning of the word as it is represented in the mental dictionary, or should it be captured by rules ('meaning postulates' as Carnap originally dubbed them in another context) for interrelating different expressions in the language? In a variety of experimental manipulations, Kintsch failed to find any evidence for an actual decomposition into more primitive elements, and he accordingly rejects the notion of analytical dictionary entries in favour of meaning postulates. There seems to be little reason to accept this conclusion.

Analytical entries may well exist, but may only be consulted in certain circumstances as, for example, when a speaker attempts to define a word, or a listener attempts to verify a sentence. Indeed, it is hard to know what empirical data would in principle decide the issue. A further problem with rules which merely relate expressions is, of course, that they provide no way of relating language to the world, no way of determining the truth or falsity of an assertion. Kintsch's theory does not

even attempt this task, not does it offer any systematic account of meaning postulates. It is a semantics without any semantics.

The one idea that does emerge from the theory is that sentences, or at least assertions, express underlying propositions. A sentence such as, "The fire destroyed many acres of virgin forest", contains four underlying propositions: (DESTROY, FIRE, FOREST); (SIZE, FOREST, ACRE); (VIRGIN, FOREST); and (MANY, ACRE). The notation presents numerous logical problems, but fortunately nothing crucial hangs upon it. Kintsch is able to predict a variety of aspects of the processing of prose simply on the basis of the number of propositions, and their gross interrelationships, that are expressed in it.

The content of the book changes abruptly in calibre in its second half, which is devoted largely to an account of a series of original experiments concerning the reading, representation, and recall, of paragraphs. This research is a sustained and sometimes ingenious attack on an intriguing psychological problem.

One final warning. This book is suitable reading only for psychologists. Logicians will be repelled by its logical inadequacies. Linguists will find little new in it. Computer scientists will be deterred by its failure to grapple with the function of a semantic theory. Psychologists, however, will find in it some stimulating experiments, written up in the detailed style of journal articles. They may also gawp at the mathematical filigree—flashy, but perhaps irrelevant—with which the author fits model to data. Meanwhile, back in the Holy Land, Jerusalem remains unconquered. □

THERE are many books on bees but few on the end product of their labours. Most books on honey are in the realms of folk law and health foods. As a result this text by a distinguished group of international specialists backed by the resources of the Bee Research Association is a landmark.

Divided into five sections with sixteen chapters the content has a potential appeal to a wide readership. Sections 1 and 3 dealing with the production and preparation of honey will appeal to beekeepers. Section 4 deals with the commercial aspects and will interest those involved in international trade and legislation.

Section 2 is the major scientific contribution covering the chemical, physical and biological characteristics and is the first attempt to put the

Honey for some

Paul Wix

Honey: A Comprehensive Survey. Edited by Eva Crane. Pp. xvi+608. (Heinemann: London, 1975.) £15.00.

material into a book where it can relate to wider issues. Section 5 has its appeal for those interested in the history and language associated with honey.

The scientists will find detailed

treatments of the chemical, physical and microscopic (pollen analysis) data, and under the brief section on biological properties every attempt has been made to separate fact from folk law. This represents a starting point for those who consider that honey still holds most of its biological secrets close-hidden.

The late 1600s woodcut with the Latin "So we the bees make honey, but not for ourselves" is a fitting tribute to the long association between man and bees. At £15 it is fairly priced although as a book of reference it might have been wise to have increased the price in favour of a paper with a finer finish. □

The Filament Fungi. Volume 1, *Industrial Mycology*. Edited by J. E. Smith and D. R. Berry. Pp. xii+340. (Edward Arnold: London, April 1975.) £12.50.

THIS is the first volume of a series devoted to the filamentous fungi and is intended to be used by senior undergraduate and postgraduate students as well as by workers involved in the pharmaceutical, chemical and food industries. The industrial applications of yeasts have been fully considered elsewhere and have not been included.

Twenty authors have contributed the sixteen chapters which constitute this volume. The backgrounds of these authors are somewhat different but it is of interest that all except four are at present working in Britain. Academics are more frequently represented than those engaged in work in industrial establishments.

No attempt is made to provide any treatment of the taxonomy of filamentous fungi or to provide assistance in their recognition. The first portion of the book deals with such fundamental aspects as structure and development, environmental control of physiology, secondary metabolism and its relationship to growth and development, strain improvement and stability and growth kinetics. The fungi are particularly prolific in the production of a wide range of secondary metabolites and since only a minority of species has so far been studied the real extent of the range of products they can produce is likely to be shown to be very much wider than is at present appreciated.

The second portion of the book deals mainly with the fungal fermentation industry. In an account of the historical development of this industry it is shown that the discovery of penicillin, followed by other antibiotics, provided the main initial impetus for the rapid expansion of the industry at a time when the background knowledge made such an expansion possible. After a discussion of commercially important secondary metabolites there are chapters dealing with the commercial production of organic acids, the transformation of organic compounds by fungus spores, industrial enzyme production, industrial exploitation of ergot fungi and oriental food fermentations. In a chapter entitled 'Submerged culture production of mycelial biomass' the modern developments in the production of protein by mycelial biomass and in the production of mycelium for use as flavouring agents are discussed. Three useful chapters on the cultivation of edible mushrooms, mycotoxins and biodeterioration and biodegradation by fungi are provided.

Not only does the text provide an up-

to-date and comprehensive treatment of the subject but the citation of a considerable number of publications at the end of each chapter is useful. The book is well produced although the few photographs are rather poorly reproduced. It represents a valuable review of an important field of study.

J. Colhoun

Mycogenetics: An Introduction to the General Genetics of Fungi. By J. H. Burnett. Pp. xiv+375. (Wiley: London and New York, 1975.) £12.50, cloth; £6.00, paper.

THIS work gives wide coverage to all the significant aspects of fungal genetics, and comprises four sections: structure and life histories of the various groups of fungi; formal genetics (genetic markers, linkage and recombination in

Fungi

meiotic and mitotic systems, extra-chromosomal elements and quantitative inheritance); population genetics (variation, selection and isolating mechanisms); industrial applications; genetics of fungal pathogenicity; and the mechanism of recombination and of gene action and regulation in fungi. Although there are no photographic plates, the text is admirably illustrated with numerous diagrams, figures, line drawings, tables and graphs. Worked examples are included and should prove valuable in understanding the complexities of, for example, the analysis of unordered tetrads and of certain aspects of quantitative inheritance.

The text is not easily read because of the extreme condensation of research publications, the profusion of technical terms and frequent directions to refer backwards or forwards to chapters or illustrations. There is also the interpolation of numerous references to original work. The introductory reader must be prepared to cope with the 'jargon' liberally sprinkling the text, as no glossary of these perplexing terms is given. Indeed, a few of the terms are not even defined in the text: for example, introgression, allopatric and sympatric.

The author must be taken to task on a number of points where I feel important omissions, ambiguities or even errors have been made. For instance, in the legend to Figure 5.4 it is mentioned that 43 out of 120 *trp-1* mutants of *Neurospora crassa* show no complementation with each other or with any of the mutants which fall into the eight complementation groups. The significance of such non-complementing mutants is not considered.

In the analysis of ordered tetrads, the

author states that the centromere distance of a locus is half the percentage frequency of second-division segregation asci for that locus because "a single cross over involves two of the four chromatids, that is, half of them". In fact, in higher diploid organisms the map unit was based directly on recombination percentage which represents the percentage frequency of "half chiasmata". In a second-division ascus, a whole reciprocal chiasma has been detected and so the percentage frequency of second-division asci is divided by two to give the centromere distance in conventional map units. The author then states that double cross overs between the locus and the centromere will be recorded as first-division segregations. This is not true because a three-strand double cross over will be recorded as a second-division ascus, as if a single cross over had occurred. The author attributes this "reduction in the real incidence of recorded cross over" to account for a maximum value of 67% second-division asci. This statement is somewhat ambiguous.

In the explanation of hybrid DNA formation during recombination between two of the four chromatids with the resulting formation of mis-matched base pairs, the author states, "Any mis-matched base pairs in the 'hybrid DNA' region are then corrected". He fails to realise that sometimes none of the mis-matched base pairs may be corrected so that no gene conversion occurs but post-meiotic segregation leads to the formation of "aberrant 4+:4m asci", with 'misplaced' spores, but in which the outside markers show normal 4+:4m segregations. Such asci are not mentioned yet they provided the key to the understanding of gene conversion. Consequently the author is, as it were, one step out in the subsequent possibilities.

Finally, the author does not mention valuable work and models proposed by Professor Olive and coworkers or the still more recent work by various researchers.

Those aspects of fungal genetics with which the author is familiar and to which he has contributed by original research, are treated in a full, interesting and up-to-date manner. To be fair to the author, he does state in the preface that he has tried to concentrate on general issues rather than to report the latest findings on any particular topic, but this method is likely to result in uneven treatment of the various aspects. He tells us also in the preface that the book is intended not only for geneticists but also for mycologists, pathologists, industrial users of fungi and biologists. I fear, however, that most of the latter types of reader will find the text turgid and tough going.

L. C. Frost

Computer checks . . .

Computer Chess. (ACM Monograph Series.) By Monroe Newborn. Pp. xii+200. (Academic: New York and London, April 1975.) \$15.00; £7.20.

1975 SEES the 25th anniversary of Claude Shannon's *Programming a Digital Computer for Playing Chess* (*Phil. Mag.*, **41**, 356; 1950), and it is an appropriate time for a review of the developments which have occurred in this field since the appearance of that classical paper.

The better computer programs in the US and USSR are now achieving standards of play which make them interesting, not only to the computer scientist, but also to the chessplayer, for, in the games of computers, the chessplayer can recognise patterns of play which are familiar among human chessplayers. Indeed, for me, a principal fascination of this book arises from the existence in computer games of traits which I would, in human players, regard as personal styles, and yet which are, in the computer, seen to be consequences of simple arithmetical processes. This is not to imply, however, that the computers have yet reached human capability, even on the chessboard, for the absence of that indefinable human quality known as 'common sense' is all too evident in the computers' deliberations. That absence is perhaps the outstanding feature of all computer chess, and makes it unmistakably different from the human variety, for, unlike the human player of comparable ability, the computer will often engage in study of local minutiae, while obvious catastrophe threatens in another sector. Examples of this amusing behaviour are frequent in the book, and will reassure the reader who fears that his mind may soon be superseded.

The full appreciation of this book requires from the reader a moderate knowledge of chess. Fortunately, there is no corresponding requirement regarding a familiarity with computer programs. Thus, in my view, the other principal success of the book lies in its presentation of the structure and mechanics of computer chess programs in a manner which is not subject to obscurity by computer software jargon. Using simple language, the author presents a short, general survey of the techniques common to most chessplaying systems, and he includes a more detailed description of one of his own, quite successful, programs.

The central part of the book consists of some 36 analysed computer games. In the analysis, both the situation as interpreted by a competent chessplayer, and the rather different interpretations made by the computers, are presented in parallel as the games proceed. The games are taken principally from American intercomputer tournaments,

and cover the period 1967-74, thus enabling the reader to assess the progress, up to quite recent times, of this rapidly evolving art.

Overall, I would say that this book represents a successful navigation of the straits between the Charybdis of obsolete tedium and the Scylla of trivial immediacy which flank this rapidly developing subject.

Nigel J. Holloway

. . . into the past

Mathematics and Computers in Archaeology. By J. E. Doran and F. R. Hodson. Pp. xi+381. (Edinburgh University Press: Edinburgh, 1975). n.p.

Few, if any, archaeologists now hold the view that 'science' has no place in archaeology, and in recent years mathematics and computing have become increasingly recognised as indispensable archaeological aids. The authors of this book rightly criticise some previous workers who have attempted to become 'scientific' by seizing on fashionable theories and forcing archaeological data into them, with a concomitant naïve and empirical use of borrowed 'hypothetico-deductive' jargon. This is an eminently readable book, refreshingly free from such jargon, which is intended for the archaeologist with no specialised mathematical knowledge, and which serves as an introduction to the subject, a critical summary of previous work, and an invaluable reference manual. It is written with simplicity but without condescension, the style being marred only by frequent confusion over the use of the first and third person and by an unnecessary proliferation of exclamation marks.

In Part I, which deals with basic archaeological and mathematical tools,

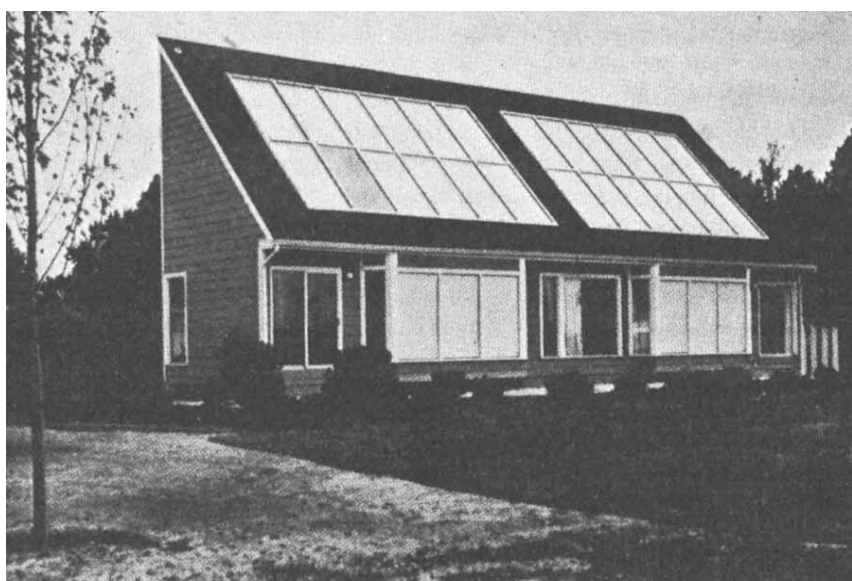
care is taken to explain fundamentals such as algorithms, vectors and functions, in terms quite comprehensible to the intelligent innumerate. Simple examples are chosen to illustrate such concepts as probability, and there is an excellent section on sampling and significance testing.

Part 2, which is devoted to data analysis techniques, presents a number of ways of dealing with the regularly recurring archaeological problem of classifying great masses of highly complex items. Reasoned, if caustic, arguments are levelled against much of the mythology of the 'New Archaeology', and at the many invalid approaches to taxonomy which have been used in the past with only partial understanding of the numerical methods involved. The section closes with a useful series of examples of data analyses, including Hodson's comparative taxonomy and seriation of the Münsingen La Tené fibulae.

Part 3, which is concerned with problems and prospects for the future, includes especially helpful sections on automatic seriation techniques and the use of computer-based archaeological data banks.

This is a volume to be thoroughly recommended, both as an introduction to first principles and as a practical comparison of the different techniques, including an assessment of their theoretical value. The general presentation of the book is of a high standard, but suffers from occasional erratic spacing and printing, together with poor reproduction of some of the simpler sketch-diagrams. At £8.00 for nearly 400 pages it is expensive but not overpriced, and it will undoubtedly remain the standard introduction to the subject for several years to come.

M. L. Shackley



Solar one, a solar electric home built by the University of Delaware. From *Energy Resources*. By Andrew L. Simon. Pp. x+165. (Pergamon: Oxford and New York, 1975.) £6.40, \$15.00, boards; £3.40, \$8.00, paper.

Synaptology

Synapses and Synaptosomes: Morphological aspects. By D. G. Jones. Pp. xiv+258. (Chapman and Hall: London, July 1975; distributed in the US by Halstead.) £14.40.

THE demonstration by Whittaker and by De Robertis in the early sixties that nerve terminals carrying synaptic complexes can be isolated in enriched fractions after controlled tissue rupture, initiated a new approach for synaptology and caused an urgent reappraisal of the nature and purity of subcellular fractions from brain tissue. Gareth Jones' monograph sets out to provide an up-to-date review of the current picture of knowledge on nerve terminals, synaptic complexes and their *in vitro* derivatives, the synaptosomes. Although the subtitle predicts a basically ultrastructural content, the text ignores this constraint and provides a biological overview of the synapse, dealing with the biochemical and physiological correlates of the observed morphology.

The final chapter gives in outline the continuing debate over the source of transmitter released in to the synaptic cleft, and deals with the vesicle hypothesis, exocytosis, and the current indications that contractile proteins may be involved in the mechanism. Another chapter describes in some detail the preparation of synaptosomes from the precise conditions of tissue rupture, the use of gradients of different types and compositions, and subfractionation of the synaptosome itself. The use of synaptosome-enriched fractions in the study of nerve terminal metabolism and of transmitter synthesis, storage and release constitutes a separate chapter. In particular it emphasises the awakening of neurochemists to the opportunities offered by the fact that synaptosomes are sealed structures, most likely generating membrane potentials and carrying a full complement of metabolic and transmitter-linked enzyme systems, fully capable of functioning in a controlled and integrated fashion for many hours *in vitro*. Moreover, they respond to depolarising stimuli with transmitter release.

The real strength and authority of the book, however, is in the two chapters on ultrastructure, reflecting the author's long association with synaptic morphology. He deals with the synapse *in situ* and the changes which may be seen in the derived synaptosome. There is a useful account of differential electron microscopic staining techniques for various synaptic constituents and components, using phosphotungstic acid, bismuth-

iodide-uranyl acetate or zinc iodide-osmium tetroxide mixtures. There is an appraisal of the ultrastructural status of an array of synaptic components including pre- and postsynaptic dense projections, coated (complex) vesicles, baskets, cytonets, reticulosomes, tetrasomes and other structures observed using new fixation, staining and other visualising methods.

The text is written in an interesting and vigorous style and is fully illustrated with copious line drawings and over 100 micrographs. It is a useful addition to the literature on synaptology and will no doubt find its way into most libraries of neurobiology provided the price is not too strong a deterrent.

H. F. Bradford

Chordate morphology

The Chordates. By R. McNeill Alexander. Pp.vi+480 (Cambridge University, April 1975.) Cloth £10.00 \$28.50; paper £4.25.

MORPHOLOGY is a dull subject, and there are only two good reasons for teaching it: either that the structures are important in the animal's way of life, or that they illuminate its evolution and relationships. But, in many universities, the systematic morphology section of zoology is now isolated in a separate course unit, divorced from the related physiological and ecological viewpoints which help to make it significant and interesting.

Those who teach such courses therefore have to find individual topics in which the importance of morphology is shown clearly, or in which its study can be used to exemplify the aims and methods of modern research. The adaptive significance of many of the gross morphological structures is clearly obvious, and is already described in many textbooks. But analyses of the mechanics of swimming or mastication, for example, have developed a great deal since most of the commonly-used textbooks of vertebrate biology were written. Alexander's book not only provides these up-to-date analyses, but also a similar range of examples of the ways in which vertebrate structures are physiologically adapted. He has succinctly set out his aims in the Preface:

"Most of the book is about animal structures, how they seem to have evolved and how they work. A great many experiments are described because it is often as interesting and worthwhile to know how an item of knowledge was obtained as to know the item itself. Since the book is about structures, many of the explanations involve physics or engineering. There are many simple calculations designed either to throw light on the dimensions of a structure, or to test the plausibility of an explanation.

There is information in this book about locomotion, respiration, blood circulation, feeding, osmotic and ionic regulation and sense organs. These topics relate well to gross structure."

Alexander has cast his net fairly widely. For example, some of the topics covered in the first 200 pages of the book are rates of water flow through gills and of gas diffusion into gills, the structure and mechanical properties of bone, the hydrodynamics of swimming, the mechanics of jaw and gill action, the physics and physiology of teleost swim-bladders, the optics of silvery reflective fish camouflage and the physiology of electric organs. Such topics are considered thoroughly and clearly, as relevant to the life of the group in question. They are dealt with at both the theoretical and the experimental level, and will help students to realise that morphology is really only a series of problems in adaptation, not all of which are, as yet, properly understood.

The book does not pretend to uniform coverage of the different chordate groups; 190 pages are devoted to fish, as against 230 for the remaining vertebrates. Criticism of this imbalance could fairly be countered by pointing out that it does not matter which group is used to illustrate a principle, as long as it is adequately illustrated. Also, since Alexander's research has been centred on fish, it is natural that he should turn to them for many of his examples. Nevertheless, it does mean that those of us whose sympathies have accompanied the tetrapods onto land will feel a little disappointed.

About half of the 214 figures were drawn especially for the book, and many of the remainder have been taken from research papers. Many will be new to those accustomed to more conventional textbooks of vertebrate morphology, for there are many figures of experimental apparatus, graphs and physiological recordings of different kinds. The diversity of sources has led to a variety of styles, and a few figures have reproduced poorly.

This book does provide a new and up-to-date functional approach and it is a valuable addition to the library of anyone designing a course in vertebrate zoology, or for students taking such a course (for whom there is a reasonably-priced paperback edition). Of course, this new approach has been at the expense of the conventional coverage of such topics as the adaptive radiation of the groups, or their relationships to one another or to geological periods and time. But for those of us who want it, this information is easily available in other books, and such omissions are a small price to pay for such a welcome, innovative book.

Barry Cox